Proceedings of the 27th Annual Aquatic Toxicity Workshop: October 1-4, 2000, St. John's, Newfoundland

Comptes rendus du 27e atelier annuel sue la toxicité aquatique du 1 au 6 octobre 2000, St. John's, Newfoundland

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PREFACE/PREFACE

The 27th Annual Aquatic Toxicity Workshop was held at the Hotel Newfoundland in St. John's, Newfoundland, October 1 to 4, 2000. The Workshop included 4 plenary presentations, 120 platform and 75 poster papers. Total attendance was 313.

This Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the Proceedings are published with the support of the Department of Fisheries and Oceans.

Le 27e atelier annuel sur la toxicité a eu lieu L'Hôtel Newfoundland, St. John's, Newfoundland les 1 au 4 octobre 2000. Le atelier a donné lieu a 4 communications lors de séances plénières, 120 exposés d'invités d'honneur 75 communications par affichage. 313 personnes ont assisté au atelier.

Le atelier a premis de poursuivre les discussions tenues annuellement au Canada sur la toxicologie aquatique et l'écotoxicologie. Ces atelier annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés l'aide du ministère des Pêches et Océans.

EDITORS COMMENTS/REMARQUES DES EDITEURS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and list of participants are also included. The papers and abstract were subject to limited review by the editors but were not subjected to full formal or external review. In most cases the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are totally those of the speakers and are neither condoned or rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Ces comptes rendus sont publiés en deux volumes, en raison de leur longeur, ils renferment le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs et une liste des participants sont aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La lonueur et la forme des communications varient parce que ces derniéres sont pour la plupart publiées intégralement. On est prié de communiquer directement avec les auteurs pour faire des remarques sur le travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont celles des conférenciers; elle ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

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PLENARY SESSION

Environmental Approach and Priorities for the 21th Century
Session Co-chairs: K. Penney and K. Coady

G. Bangay. Regional Director General, Atlantic Region, Environment Canada.

D. Burley. Manager of Environmental Affairs, Canada Newfoundland Offshore Petroleum Board.

K. Dominie. Assistant Deputy Minister-Environment, Newfoundland Department of Environment and Labour.

Dr. C.R. Lucas. Dean of Science, Memorial University of Newfoundland.

PLATFORM SESSION

Oil and Gas Environmental Effects Monitoring

Session Co-chairs: M.R. Anderson, P.G. Wells and U. Williams

Sable Offshore Energy Inc. Offshore Environmental Monitoring Program Results 1998-1999. S.L. Belford[1] and G. Hurley[2]. [1]Jacques Whitford Environment Ltd., Dartmouth, NS; [2]Sable Offshore Energy Inc., Halifax, NS.

Sable Offshore Energy Inc. (SOEI) is required to develop and conduct an Offshore EEM Program as a condition of the Development Plan approval granted by CNSOPB. According to SOEI's Development Plan the EEM program was designed to test impact predictions made in the EIS and collect data for environmental management decisions. The Tier I EEM Program focuses mainly on the activities at Venture, Thebaud and North Triumph. Drilling was conducted at all three locations by December 31, 1999. Baseline information was collected in 1998 and 1999. The environmental attributes chosen for the Offshore EEM Program were based on VEC's identified during the EIS process and attributes identified by SEEMAG and the EEM study team. The selected attributes include: water quality, suspended particulate matter (SPM) in the benthic boundary layer (BBL), sediment quality (chemistry and toxicity), benthic habitat and megafaunal community, shellfish body burden and taint, marine mammals and sea birds. The sampling design consists of a radial grid with eight axes. Sampling is conducted along transects at increasing distances from each platform (250 m to 20 km). Water samples collected on a transect (250-20,000 m from the drilling platform) and in profile along the axis of the prevailing current did not contain detectable levels of hydrocarbons during drilling phase surveys. An apparent plume was detected only once out to 500 m from Venture during the Fall 1998 survey. There were no significant differences in either SPM or Ba concentrations in the BBL around the three drilling platforms that can be attributed to drilling activities. Bentonite was not present as a component of the SPM. Drill cuttings piles were visible within 70 m of the discharge pipe. Elevated levels of total petroleum hydrocarbons (TPH) and Ba were generally found at 250 and 500 m from the drilling platforms and were short-lived. Dispersion or burial appeared to occur within a six month period and is likely attributed to sediment transport. Sediment toxicity was observed in amphipod mortality testing and in echinoid fertilization testing at stations throughout Venture, Thebaud and North Triumph fields for the baseline surveys. Microtox® testing on sediment samples from all fields during baseline and drilling phase surveys showed no toxicity. Amphipod toxicity at 250 m at Thebaud and North Triumph corresponded with elevated TPH levels. The dominant epifauna in the Venture and Thebaud fields are sand dollars, and the dominant epifauna at North Triumph are brittle stars with sand dollars. No obvious effects on benthic fauna or habitat are evident. Scallops collected from natural beds in the project area, showed low levels of aliphatic hydrocarbons in baseline and drilling phase surveys. No taint was detected in sensory evaluations. In mussels moored at Venture, small amounts of hydrocarbons were detected from 500 m to 13 km and in the control sites. The concentration of hydrocarbons detected in mussel tissues was not solely attributed to hydrocarbon releases from discharged drilling muds. High peaks of pristane are evidence of healthy feeding mussels. The data generated from observations of marine mammals and sea birds made by trained observers provided no evidence of avoidance or attraction to the project area by these animals. For the ongoing offshore EEM the practicality, logistics and analytical limitations of some parameters should be reviewed, as well as modifications of sampling locations and effort.

Terra Nova's Environmental Effects Monitoring Program. U. Williams[1] and M. Murdoch[2]. [1]Terra Nova Development, St. John's, NF; [2]Jacques Whitford Environment Limited, St. John's, NF.

The Terra Nova oil field is situated on the Grand Banks, about 35 km southeast of the Hibernia oil field

and about 350 km east-southeast of St. John's, Newfoundland. The oil field is being developed using an FPSO (Floating Production Storage and Offloading) facility with a drill rig being used to drill the production wells. As a protection against icebergs, production wellheads will be located in four glory holes. Crude oil will be transported to the FPSO via trenched flowlines connected to flexible risers. First Oil is anticipated in the first quarter of 2001. Terra Nova's EEM program is conducted to evaluate impact predictions, provide early warning of potential future challenges, provide information for making sound environmental decisions, and provide the basis for technological improvements. This presentation will outline Terra Nova's EEM design and describe the process used in reaching that design. Drilling and production discharges were considered in developing the sampling design and identifying monitoring variables. Baseline data were collected in 1997 and form the foundation upon which the EEM program is structured. This is the first year for implementing the EEM program.

Ecotoxicology of Oil Development in the Marine Environment: Past and Present. J.F. Payne. Department of Fisheries and Oceans, St. John's, NF.

The offshore oil and gas industry which is rapidly developing off the east coast of Canada has become a focus of concern for potential effects on fisheries and the environment. For instance, with respect to the Grand Banks of Newfoundland, the Terra Nova, Hibernia and White Rose Fields are situated on important fishing grounds in a relatively small triangle on the North East edge of the Banks. The potential for cumulative impacts on biota around individual rig sites as well as the potential for more broad scale impacts from "combinations" of discharges of drilling muds, production waters and displacement waters by various rigs over the next few decades will likely be an ongoing concern. What will be the scale and nature of impacts at individual petroleum development sites? Overall, the majority of information available from field and laboratory studies suggests that offshore impacts will likely be minimal with little potential for cumulative impacts beyond individual rig sites. As noted by others, it is also not unreasonable to suggest that fishery closure zones around some rig sites could act as important nursery areas. General statements should always be approached with caution and it is very important to point out that uncertainties about the effects of oil development still exist, particularly with regard to the potential for chronic ecotoxicological effects be they in relation to the size of impact zones for effects on fish health and productivity, sediment toxicity, or for instance the potential for larval effects or localized change in the structure of plankton communities around any rig sites with "prolonged" stagnant water regimes. Recent, ecotoxicological studies, including chronic effects studies we have carried out under the Program on Energy Research and Development will be reviewed. These will be discussed in relation to knowledge gaps which need to be addressed further through laboratory and/or monitoring studies. With respect to monitoring studies around individual rig sites, different approaches could be required due to differences in ecotoxicological potential of discharges, species or resources at risk, habitat types, and current regimes.

Ecosystem Level Effects of Offshore Platform Discharges- Identification, Assessment and Modelling. R.B. Rivkin[1], R. Tian[1], M.R. Anderson[2], J.F. Payne[2] and D. Deibel[1]. [1]Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF; [2]Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, St. John's, NF.

ABSTRACT

The discharge of dissolved and particulate material at offshore platform sites has the potential to influence seawater chemistry and the biota in the marine environment. Studies that measure the concentrations of platform-derived inorganic and organic compounds in seawater and the benthos, or bioassay these compounds have certainly contributed to the development of regulatory guidelines for platform-related activities. Unfortunately, by neglecting the complexity of marine ecosystems, these

studies generally provide little insight into both long-term and far-field effects of these offshore discharges. There are several approaches to address these effects. Although the direct measurement of the long-term responses of pelagic and benthic communities is sometimes feasible, dynamic models of ecosystem processes provide insights into complex community level interactions and responses to natural and anthropogenic disturbances. We conducted a modelling sensitivity analysis to assess the perturbations in food web structure and energy flow due to the discharge of produced water. The model predicts large and significant increases in primary and secondary production and sedimentation fluxes over large spatial domains (100 km^2) in response to produced water-derived NH_4 and dissolved organic carbon inputs. The food web structure shifted from a diatom-copepod food chain to one dominated by small autotrophs and heterotrophs and microbial grazers. The resulting shift in food web structure redirected energy flow towards the benthos with a concomitant increased flux of carbon and contaminants to the sea floor.

INTRODUCTION

Produced water is the largest volume waste stream from exploration and oil and gas production activities (Stephenson 1992). Over the operational lifetime of an offshore field, the produced water that is discharged into the ocean can exceed, by 10-fold, the total production of hydrocarbons. Produced water typically consists of formation water (water associated with the oil reservoir) and injection water (water used to maintain pressure and oil production; e.g. Davies and Kingston 1992). The formation-water component of production water is a brine which derives its salinity from the major ions found in seawater (GESAMP 1993). However, depending on the nature of the oil formation, produced water also contains a number of metals, hydrocarbons, and inorganic and organic constituents that can potentially influence marine organisms and thus impact oceanic habitats. The chemical composition of produced water is highly variable both over time within a particular field (i.e. as the well ages) and among oilfields (Goodman and Troake 1984, Davies and Kingston 1992, Allen and Robinson 1993). As an oilfield ages, there is an increase in the amount of produced water generated during the oil production process until eventually the level of produced water exceeds the amount of oil produced (Farestveit *et al.* 1996). Although site-to-site differences and age-dependent changes in the composition of produced water make the extrapolation of results among studies and sites difficult, the use of models may allow better and more accurate predictions of the potential environmental impact of produced water.

Models are tools that define relationships among variables and therefore facilitate and enhance our understanding of complex patterns and interactions. They can be simple "conceptual models" which identify the associations among model components (i.e., Rivkin *et al.* 1996) or complex "mathematical models" which describe and quantify the relationship (Fasham *et al.* 1990, Tian *et al.* 2000a). Models therefore facilitate the development of insights into mechanistic relationships, provide a framework for interpreting observations and can lead to diagnostic or prognostic predictive capabilities. In the case of produced water, models allow the connection between source conditions and the receiving environment. To date, numerous models have been developed to describe the distribution and dispersion of hydrocarbons, and other toxic compounds (Brandsma and Smith 1996, Murray-Smith *et al.* 1996, Rye *et al.* 1996), and the effect of acute and chronic exposure and the associated environmental, health and safety risks of produced water and its components (Øljord *et al.* 1996, Reed *et al.* 1996, Meinhold *et al.* 1996 and references cited therein). However, to our knowledge, the effect of the nutrients, such as dissolved organic carbon, volatile fatty acids, ammonia, phosphorous, etc. found in produced water has not been considered in modelling studies. In this paper we used an ecosystem model developed for and successfully applied to several coastal and oceanic sites (Tian *et al.* 2000a,b,c) to examine the effects of NH_4 and dissolved organic carbon from produced water on primary and secondary production, and food web structure, for an ecosystem typical of that on and near the Grand Banks of Newfoundland, where extensive offshore production facilities are being developed or planned.

METHODS

We reviewed the published literature for information on produced water discharge rates, composition and environmental effects. Most studies were from sites in the North Sea and the Gulf of Mexico, however other sites (e.g., California, Australia, etc.) were also included. Using our ecosystem model, we assessed the effect of the addition of NH_4, an important and often limiting nutrient for phytoplankton growth and volatile fatty acids (VFA), a highly labile substrate for bacterial growth. Both total organic carbon (TOC) and VFA are readily useable by marine bacteria. In biodegradation studies (Flynn *et al.* 1996), the specific turnover rates for TOC and VFA were 0.65/d and 0.27/d, respectively. Although many of the studies reviewed here reported TOC and particulate organic carbon (POC), estimates of dissolved organic carbon (DOC) from the difference between TOC and POC could be subject to large errors. Hence, we used VFA (i.e. DOC) in our model, although we recognize that this underestimates the actual input produced water-derived bacterial substrates.

The ecosystem model has been described previously (Tian *et al.* 2000a,b). This 1-D model is driven by vertical mixing with real meteorological forcings (i.e. vertical mixing coefficients vary continuously with depth) and includes both the herbivorous diatom copepod food chain and the microbial food web (Fig. 1). The simulation is tightly constrained by detailed biological observations that include measurements of production, export and food web structure collected in Conception Bay, Newfoundland. This model-run represented the natural "unperturbed" ecosystem. We then sequentially added NH_4, DOC and NH_4 + DOC fluxes and analyzed the ecosystem response. The fluxes were computed from realistic produced water discharge rates (Hodgins 1994) and literature values for NH_4 and VFA (Table 1). Ecosystem simulations were carried out for nutrient fluxes diluted over two spatial scales, 25 km^2 and 100 km^2. We assumed that the mixing of produced water was instantaneous over specified areas and horizontal advection was negligible.

RESULTS AND DISCUSSION

The discharge rate and composition of produced water vary among sites, and well activity and age. Although there is no consistent pattern in discharge rate (i.e. Gillibrand *et al.* 1995, Røe *et al.* 1996), the chemical composition shows several commonalties among sites. The components commonly found are dispersed and soluble oil, heavy metals, radionuclides, treating chemicals, salt, and dissolved organic and inorganic nutrients (Middleditch 1984, Stephenson 1992, Ynnesdal and Furuhold 1994). Because toxicity is dose and concentration dependent, the potentially toxic effect of these compounds is thought to be relatively small due to rapid dilution of the produced water (Sommerville *et al.* 1987, Flynn *et al.* 1996 and references cited). Produced water also contains very high concentrations of essential and often growth limiting nutrients, such as NH_4 and dissolved bacterial substrates such as organic carbon compounds and fatty acids (Table 1). The potentially stimulatory effect of these constituents on marine planktonic communities has received very little attention (Gamble *et al.* 1987). Nutrient concentrations (Table 1) in produced water are often 10^3- to 10^8-fold greater than in receiving water and this could potentially result in an enhancement of production, and alterations in food web structure and biogeochemical fluxes.

In the North Sea, where there is extensive offshore oil and gas development, the release rates and composition of produced water have been well studied (Tibbets *et al.* 1992, Flynn *et al.* 1996, Murray-Smith *et al.* 1996, Røe and Johnsen 1996, Gillibrand *et al.* 1995 and references cited therein). Based upon published information on produced water discharge rates and chemical composition, we can estimate the basin-wide input of the nitrogenous nutrients. The annual produced water discharge is 1.8 to 3.3 x 10^8 m^3/y (Gillibrand *et al.* 1995, Røe and Johnsen 1996), which is equivalent to ~0.5-1.0% of the total fluvial input of 380 x 10^9 m^3/y into the North Sea (Howarth *et al.* 1996). If we assume that the NH_4 concentration in produced water is 75 mg/L

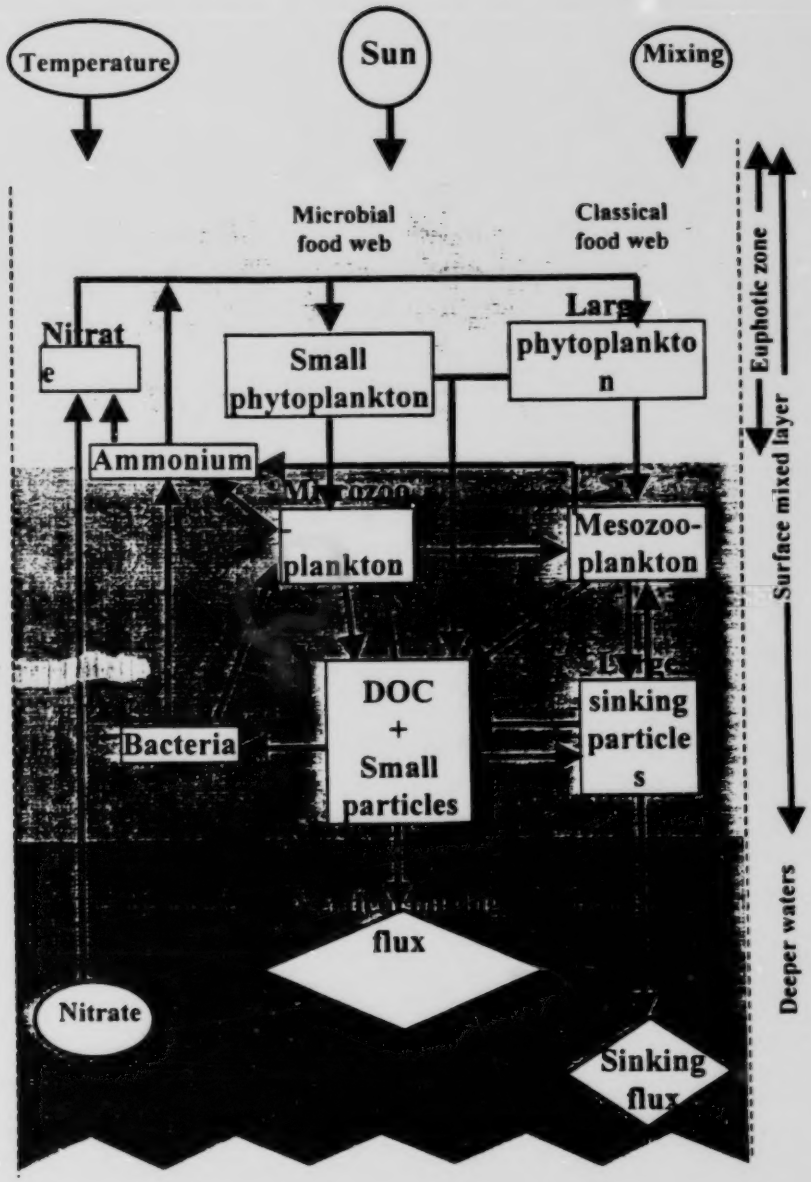

Fig. 1. General ecosystem model showing major state variables (NO_3, bacteria, small and large phytoplankton, micro- and mesozooplankton, dissolved organic carbon [DOC] and small detritial particles and large sinking particles), and forcing (temperature, irradiance and mixing). The arrows show the major fluxes between variables.

(Table 1 and Tibbets *et al.* 1992), NH_4-N input is 1.5 to 2.6 x 10^{10}g N/y. The produced water derived NH_4 concentration can be computed from the area of the North Sea (575,300 km^2) and the mean depth (73.5 m). If we make the assumption that the produced water is rapidly diluted over the entire volume of the North Sea (i.e. 42,294 km^3), the annual input of NH_4 is computed to be 25-45 µMol/year. This is certainly a very conservative estimate since the assumption of instantaneous mixing over the entire water column is overly simplistic. The planktonic community's response to an NH_4 flux of this magnitude, especially in an N-limited marine environment, could be dramatic and lead to changes in the rates of primary and secondary production and patterns of carbon and energy flows. This calculation demonstrates that produced water can introduce large and "ecologically significant" quantities of nutrients into the marine habitat.

Table 1. Summary of literature review (1985-1999) on concentrations (mg/L) of ammonia (NH^4), total organic carbon (TOC) and volatile fatty acid (VFA) concentrations in produced water.

	NH_4 mg/L	TOC mg/L	VFA mg/L
Maximum	300	1,500	4,900
Minimum	10	15	20
Average	75	300	370

Using our ecosystem model, we quantified the planktonic community's response to produce water-derived fluxes of NH_4 and DOC for the Hibernia site on the Grand Banks of Newfoundland. Nutrient fluxes were estimated for 25 and 100 km^2 areas using the predicted produced water discharge rate of 20,000 m^3/d (Hodgins 1994) and the literature average NH_4 and VFA concentrations of 75 and 370 mg/L, respectively (Table 1). The dominant component of VFA is acetic acid (Tibbetts *et al.* 1992), we therefore conservatively estimated that carbon is 40% of the weight of the VFA input into our model. We assumed that the mixing of produced water was instantaneous over specified areas. This resulted in fluxes of 3.3 and 0.83 mMol NH_4-N m^2/d and 10 and 2.5 mMol DOC-C m^2/d for the 25 and 100 km^2 areas, respectively. Vertical mixing is forced by local meteorological events assuming a constant shear term due to tidal mixing. Thus the mixed layer depth is generated and propagated by model and varies from ~10 m in summer to the bottom (~85 m) during winter. The model's initial condition was parameterized and tested using data collected from a coastal Newfoundland site and sensitivity analysis was used to assess the ecosystem response to an external nutrient flux from produced water.

The model predictions are shown in Fig. 2. The model-estimated fluxes are size-dependent phytoplankton production (PP; Fig. 2A), bacterial production (BP; Fig. 2B) as a proxy for microbial food web activity, mesozooplankton production (ZP; Fig. 2C) and sinking biogenic carbon (SF; Fig. 2D). Relative to the initial condition, i.e. no produced water input, over the 25 km^2 area, the discharge of produced water increased PP, BB, ZP and SF by 1.4- to 2.2-fold, 1.4- to 1.7-fold, 1.3- to 1.5-fold and 1.5- to 2.8-fold, respectively. Over the 100 km^2 area, the discharge of produced water increased PP, BB, ZP and SF, relative to the initial condition, by 1.1- to 1.4-fold, 1.2- to 1.4-fold, 1.1- to 1.3-fold and 1.1- to 1.3-fold, respectively. The addition of nutrients from produced water resulted in a shift in food web structure and change in the pattern of biogenic carbon flux, as well as increasing primary and secondary productivity. For example, the increase in PP was due almost entirely to changes in production by small (<5.0 µm) phytoplankton (Fig. 2A). This has profound food web implications since crustacean zooplankton, such as copepods, do not efficiently ingest small cells. This food-web effect can be seen as a decrease, relative to the initial condition, in the ratio of ZP:PP. This index of carbon

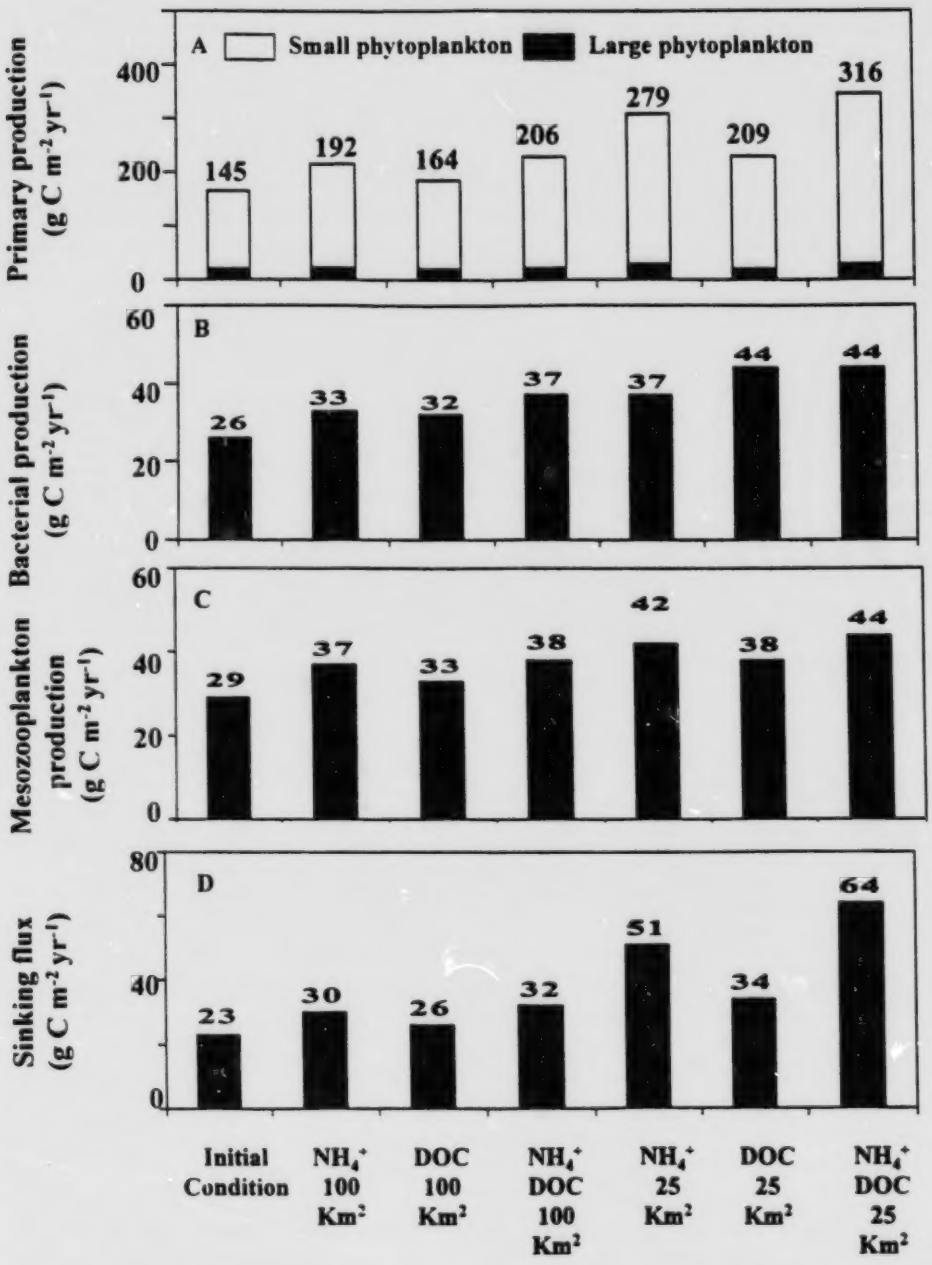

Fig. 2. The model-estimated fluxes of small and large phytoplankton production (A); bacterial production (B); mesozooplankton production (C); and sinking biogenic carbon (D) for the initial condition, (i.e. no produced water input), and for the input of produced water-derived NH_4 and DOC (i.e. volatile fatty acids) integrated over 100 and 25 km^2 spatial domains. See text for the detailed discussion of model assumptions and nutrient fluxes.

flux declined from 0.20 for the initial condition, to 0.19 and 0.15 for the 100 and 25 km^2 areas, respectively and suggests that, at least over 25 km^2 PP is being channelled into the microbial food web or is being exported as aggregates rather than supporting a herbivorous food chain leading to enhanced pelagic fisheries. The modelled results for sinking flux support this scenario: The ratio of SF:PP (i.e. *e*-ratio) increased from 0.15 for the initial condition, to 0.16 and 0.18 for the 100 and 25 km^2 areas, respectively.

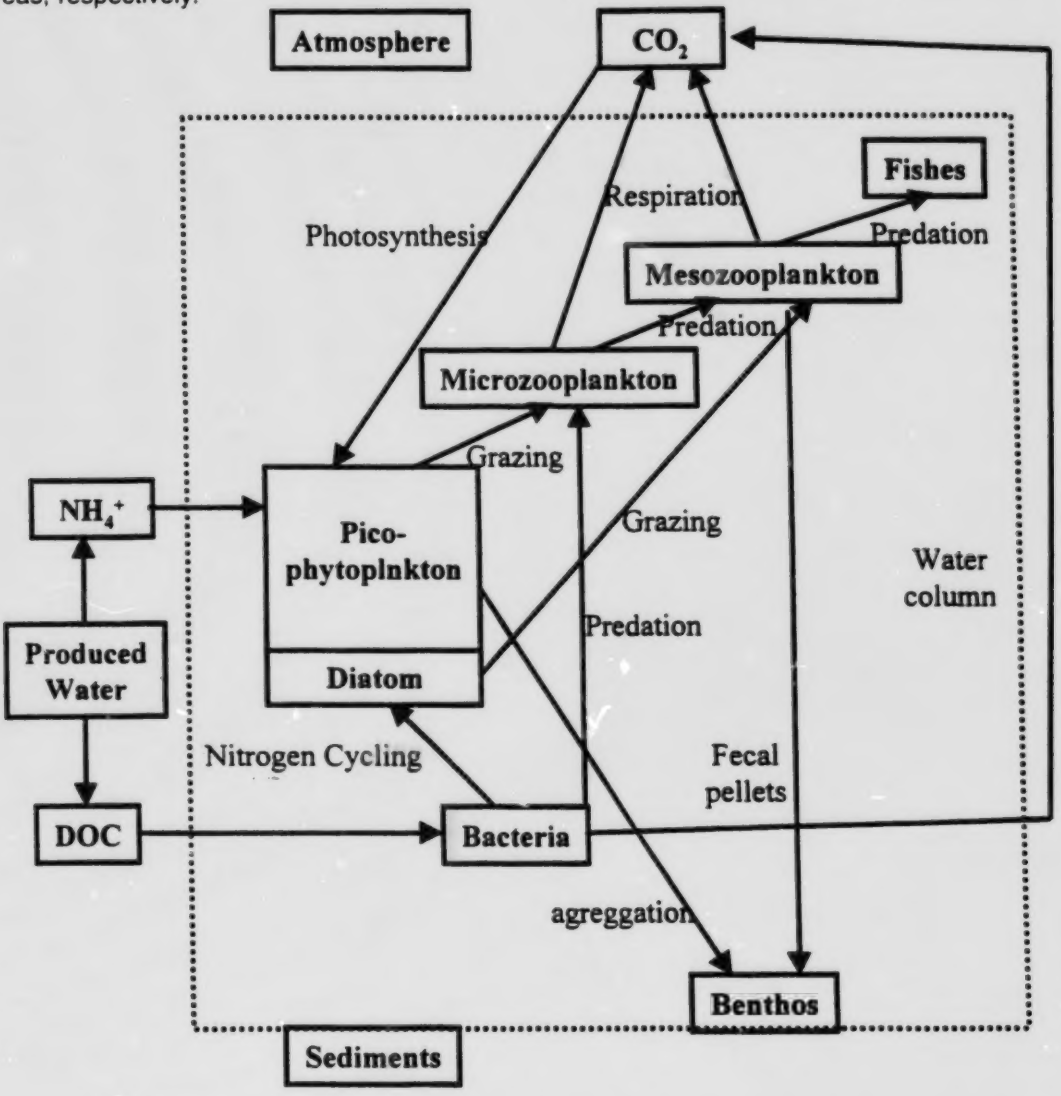

Fig. 3. Simple conceptual model of the food web on the Grand Banks of Newfoundland and the effect of produced water-derived inputs of NH_4 and DOC (i.e. VFA) on energy flow. Although the model predicts a significant increase in primary and secondary production, there is a disproportionate enhancement of small-celled autotrophs and heterotrophs. This leads to a reduction in the fraction of primary production being ingested by mesozooplankton and a larger fraction of primary production being remineralized to CO_2 and vertically exported. The increased remineralization may contribute to enhanced sea-air CO_2 flux. The increased vertical flux could lead to a higher benthic biological oxygen demand and rapid transport of produced water-derived contaminants to the benthos where they could accumulate to high concentration and be readily available to benthic organisms.

CONCLUSION AND IMPLICATIONS

Ecosystem models can provide insights into complex community level interactions and responses to natural and anthropogenic disturbances. In this study we adapted a model, which was parameterized using a data from Newfoundland coastal waters, to predict direct and indirect ecosystem level effects of produced water-derived nutrients on the community structure and rates and patterns of biogenic carbon flux. The model predicts a significant increase in primary and secondary production over large spatial (25-100 km^2) and temporal (annual) scales. However the nutrient input disproportionately enhances the production of small-celled autotrophs and heterotrophs (Fig. 3). As a result, the fraction of PP that is exported to mesozooplankton (and consequently higher trophic levels) decreases, and the fraction vertically exported (and therefore transported to the benthos) increases.

The discharge of produced water into productive marine waters has two important implications: First, the channelling of PP into the microbial food web or to vertical exported decreases the fraction available to planktonic mesozooplankton. Since mesozooplankton are the primary food for larval and juvenile fish, this could change the balance between pelagic and demersal fisheries. Second, the increased vertical flux (mainly highly reactive phytoplankton aggregates) could lead to an increase the benthic biological oxygen demand. Moreover there will be a rapid transport of produced water-derived contaminants (e.g. trace metals, hydrocarbons, etc.) to the benthos where they could accumulate to very high concentration and be readily available to benthic organisms. This effect would be significant over large spatial scales since planktonic organisms accumulated these contaminants and sinking aggregates scavenge trace metals and other contaminants from the water column (Vinogradov 1953, Fisher *et al.* 1983, 1984, 1991).
When properly parameterized, ecosystem models can be used to predict direct and indirect ecosystem level effects (e.g., regime shifts, development of alternative food webs) that cannot be inferred simply from knowledge of contaminant distributions and classical toxicology. As such, this approach has the potential to identify mechanisms for observed results which in turn can lead to an understanding of ecosystem level effects of offshore oil platform discharges on both pelagic and benthic communities and thus provide insights for proactive mitigation strategies.

ACKNOWLEDGEMENT

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Requirements and Considerations for the Development of Offshore Field Programs. C. Hollett and S. Whiteway. Jacques Whitford Environment Ltd., St John's, NF.

Field program preparation is an integral component in the successful execution of any field program. The up-front planning and preparation can directly impact the quality and validity of the information collected during these programs. Newfoundland's geographical nature provides unique challenges for

the preparation and execution of field programs with respect to location accessibility; limited major population centers and related infrastructure; and weather conditions that can be variable and adverse. Areas within the Labrador region of the province and a thriving offshore oil industry both demonstrate the unique challenges our province presents to the preparation and execution of field programs from a logistical, quality and administrative perspective. This presentation will focus on the attributes and considerations required for the successful execution for offshore field programs. The factors that result in the successful execution of offshore field programs are discussed in detail and include the challenges and obstacles to the execution of offshore programs; team characteristics; client-consultant relations; communications; initial equipment selection; logistical considerations including sample platform, platform layout, equipment modifications, pre-cruise preparations, sample handling and storage; contingency planning; quality planning and safety considerations.

The Necessity to Monitor the Impacts of Offshore Oil Platforms on Seabirds. F.K. Wiese[1], W.A. Montevecchi[2], G. Davoren[2], F. Huettmann[3], A.W. Diamond[3] and J. Linke[3]. [1]Department of Biology, Memorial University of Newfoundland, St. John's, NF; [2]Biopsychology Programme, Memorial University of Newfoundland, St. John's, NF; [3]Atlantic Cooperative Wildlife Ecology Research Network, University of New Brunswick, Fredericton, NB.

Seabirds, are attracted to large offshore structures such as oil drilling platforms. Possible causes for this attraction include the physical structure, increased concentrations of food, night lighting and flaring. Although concerns about this attraction have been referred to frequently in environmental assessments of offshore oil and gas exploration, these impacts have not been documented systematically or quantified. Concerns in the Northwest Atlantic are unique in that huge concentrations of planktivorous seabirds, many of which are strongly attracted to artificial light sources move through the region in autumn (storm-petrels), winter (dovekies, murres), spring and summer (shearwaters). Birds attracted to offshore platforms are consequently at increased risk of oiling should hydrocarbons, including drilling fluids and those in production waters, escape from the drilling platform, and as well from ingesting potentially contaminated food items around the platform. Most of the birds that occur at sea in the study area throughout the year are long distance migrants and do not breed in Canada. Regional hydrocarbon development could therefore affect both local and global breeding populations of seabirds. There is an urgent and pressing need to have trained, dedicated, independent observers monitor this potential impact in order to quantify and determine the nature, timing and extent of bird mortality caused by these structures. Recommended monitoring systems will be presented.

Potential Toxicity of Produced Water from the Canadian Offshore Oil and Gas Industry. K. Lee[1], G. Wohlgeschaffen[1], K. Azetsu-Scott[2], S. Niven[2], P. Yeats[2] and J. Dalziel[2]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

With the development of Canada's offshore oil and gas reserves, large volumes of produced water may be discharged into the ocean. Produced waters may contain a number of metal and organic constituents of environmental concern including: [1] hydrolysis metals; [2] heavy metals; [3] petroleum hydrocarbons; [4] nutrients; [5] radionuclides; and [6] treating chemicals. A regional assessment to identify potential produced water impacts is required as the composition from different sources and formations can vary by orders or magnitude on a constituent-specific basis. Preliminary studies were conducted with samples of produced water from an offshore production well on the Scotian Shelf. In addition to petroleum hydrocarbons, elevated concentrations of Al, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni and Zn were measured in total, particulate, and dissolved phases. These heavy metals were transformed from a dissolved to particulate form in a period of hours under oxygenated conditions. The majority of these precipitates flocculated to large particles with a sedimentation rate >100 m/day. Positively

buoyant particles associated with dispersed oil droplets were also observed. Microtox® assay results illustrated an increase in produced water toxicity with time following its discharge into the sea. In general toxicity levels peaked after samples were oxidized for one to two days and were sustained at significant elevated levels for more than a week. Future risk assessment models should consider the ecological significance of observed chemical processes that mediate the transport of heavy metals as well as the dispersion and weathering of residual hydrocarbons.

Produced Water from Offshore Hydrocarbon Drilling and Production: Effects on the Early Life Stages of Haddock (*Melanogrammus aeglefinus*), Lobster (*Homarus americanus*) and Sea Scallop (*Placopecten magellanicus*). K. Querbach[1], G. Maillet[1], P.J. Cranford[2], C. Taggart[1], K. Lee[2] and J. Grant[1]. [1]Department of Oceanography, Dalhousie University, Halifax, NS; [2]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Acute and chronic effects of exposure to produced water (PW) from offshore drilling were quantified for the early life stages of haddock (*Melanogrammus aeglefinus*), lobster (*Homarus americanus*) and sea scallop (*Placopecten magellanicus*) in terms of survival, growth and fertilization success. During 96 h exposures to 0-25% PW, yolk-sac haddock larvae, feeding stage-I lobster larvae (fed), and scallop veligers each displayed significant reductions in survival at 10 and 25% PW. The average size of scallop veligers was significantly reduced after exposure to 10 and 25% PW. Scallop fertilization success was significantly reduced at all concentrations ≥1%. During 18 d chronic exposures to concentrations of 0-10% PW, significant reductions in scallop veliger survival and size were observed in the 10% treatment. Chronic exposure of the diatom, *Thalassiosira pseudonana* to 10% PW resulted in a significant reduction in physiological condition though there was no effect on chlorophyll-a concentration. These results show the possibility of using resource-based species in toxicological evaluations even though the typically high mortality for these organisms exceeds the established lab-based criteria for assessing biotest results.

A New EEM Approach for Oil and Gas Based on Hazard and Risk Assessments. I. Use of Scallops to Monitor the Potential Hazard of Chronic Discharges of Synthetic Drilling Mud. S.L Armsworthy, P.J. Cranford, K. Lee and K. Querbach. Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

An environmental concern related to offshore oil and gas production is the consequences of long-term exposure of drilling wastes to resident organisms. While synthetic oil-based drilling muds (SBM) were developed in response to laboratory and field observations of significant environmental impacts associated with the discharge of mineral oil-based muds (OBM); recent Canadian regulatory guidelines have effectively prohibited the discharge of SBM contaminated well cuttings as a precautionary measure. Owing to the operational advantages of their use, an objective assessment of SBM discharges is warranted given that the majority of SBM is recycled while all used water-based mud (WBM) is discharged. Sea scallops (*Placopecten magellanicus*), which are highly sensitive to drilling wastes, were exposed under environmentally relevant conditions in the laboratory for approximately two months during summer and winter to different types and concentrations of used SBM formulations. Toxicity was assessed from chronic lethal and sublethal (somatic and reproductive tissue growth, and physiological condition) effects. Sea scallop survivorship in all SBM treatments (0.07-9.6 mg/L) was similar to the controls. Tissue growth was significantly impacted by the Novaplus® and IPAR-3® treatments at 0.07 and 1.0 mg/L during winter and summer, respectively. Growth reductions resulted primarily from reduced energy intake through feeding (clearance) and digestion (absorption), but increased energy losses to respiration and excretion also contributed to reduced growth. Clearance rate was particularly sensitive to suspended SBM and an EC_{50} value between 0.2-0.5 mg/L was indicated. The toxicity of the SBM was relatively low compared with drilling fluids containing OBM, but

was greater than for WBM. Results suggest that utilization of a best practicable discharge limit for SBM on cuttings could have less impact on scallop stocks than predicted from the discharge of WBM.

A New EEM Approach for Oil and Gas Based on Hazard and Risk Assessments. II. Numerical Model Assessment of the Potential Risk to Scallop Stocks of Drilling Wastes. P.J. Cranford, D.C. Gordon, C.G. Hannah, J.W. Loder, T.G. Milligan and D.K. Muschenheim. Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

A numerical benthic boundary layer transport model (BBLT) was developed to provide estimates of the suspension, drift, dispersion and concentration of drilling wastes released during oil and gas exploration and production drilling on the eastern Canadian continental shelf. Example predictions are presented for hypothetical drilling sites utilizing water-based drilling muds on Georges Bank. Simulations predict that highest near-bottom concentrations of drilling mud would occur in the relatively deep Side region (>100 m) as a result of relatively low suspension, dispersion and drift. Lowest concentrations would occur in the central Mixed region (<65 m) because of high dispersion, while intermediate concentrations would occur in the Frontal region. Laboratory experiments show that adult scallops are highly sensitive to drilling mud, and the near-bottom concentration time series from BBLT simulations provide a basis for estimating impacts. The region of greatest potential impact on scallop growth is the Side region where mud concentrations from the hypothetical release scenario are predicted to prevent scallop growth for 2-40 d depending upon the settling velocity used and area over which results are averaged. Scallop stocks in this region are relatively small but dense aggregations are found in some areas. Growth losses in the Frontal region, which has the densest scallop stocks, are predicted to be more localized and confined to a range of 0-15 d. Predicted growth loss in the central Mixed region is predicted to be negligible (<2 d). A quantitative analysis of the potential risks to scallop stocks associated with the discharge of water- and synthetic oil-based muds will be presented.

A New EEM Approach for Oil and Gas Based on Hazard and Risk Assessments. III. Bivalves as Sentinel Organisms for Monitoring Environmental Impacts from Production Drilling Wastes. P.J. Cranford, S.L. Armsworthy and K. Lee. Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

New *in situ* methods and technologies using bivalve filter-feeders as sentinel organisms for relating contaminant concentration and bioavailability to the onset of biological effects are being developed and tested for potential incorporation into EEM programs at offshore oil and gas production fields. Two new EEM tools have been field tested at Hibernia to assess their capacity to detect the spatial and temporal extent of the cumulative and relative impacts of different drilling fluid formulations, disposal regimes and waste dispersal processes. These include the construction of *in situ* moorings containing caged Icelandic and sea scallops and mussels that are placed on the seabed at various locations relative to the Hibernia platform. Data on lethal and sublethal (morphology, somatic and reproductive growth, condition index and nutrient state) impacts and contaminant body burden are being used to determine the most sensitive biomarkers for EEM, to quantify dose-response relationships, and to help define the spatial extent of benthic impacts from drilling activities. Observed impact zones will be compared with results from standard EEM approaches including benthic community analysis and sediment toxicity bioassays. The second EEM tool consists of an *in situ* biological effects monitoring system (HABITRAP) that continuously measures bivalve food utilization, which is directly related to growth and reproductive output of a resident resource species (Icelandic scallops). Feeding rate was shown to be inversely and closely related to the barium content (a tracer of drilling wastes) of sedimenting particles. Results obtained on short-term biological responses will provide insight into the relative impact of specific drilling practices (e.g., bulk dump of water-based mud). These practical,

sensitive and cost-effective EEM approaches and technologies may provide information on the environmental effects of drilling and production activities to support the development of regulatory guidelines that reduce costs to industry and mitigate environmental concerns identified through hazard and risk assessments.

Comparison of the Abilities of Effluents from Two Refineries and a Municipal Wastewater Plant to Cause Estrogenic and Anti-Estrogenic Responses in Rainbow Trout. J.P. Sherry[1], S. Munro[2], T. Moran[3], T. Kierstead[2], P.-D. Hansen[4] and B. Hock[5]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Sarnia-Lambton Environmental Association, Sarnia, ON; [3]Pollutech Enviroquatics Ltd., Point Edward, ON; [4]Department of Ecotoxicology, Technical University of Berlin, Berlin, Germany; [5]School of Botany, Technical University of Munich, Freising, Germany.

The egg yolk protein vitellogenin (Vg) has gained acceptance as an indicator of exposure to environmental estrogens. Data from some previous experiments in our laboratory indicated that the final waste water from an Ontario refinery may have the ability to induce Vg in rainbow trout and in cultured trout hepatocytes. Those early results were obtained from a 15 d static renewal bioassay with limited replication (n=5), in which the effluent in the individual aquaria was partially (33.3%) renewed on alternate days. For the present study we collected fresh wastewater daily from two refineries, a municipal wastewater plant, and a reference upriver site. The ability of the wastewater to induce Vg was assessed in a 21 d static renewal bioassay (n=15) in which the exposure solutions were completely changed on a daily basis. Fish (n=30) were caged simultaneously in the recipient water close to the point of discharge from each facility, and at the reference site. Half of the caged fish were primed with 17β-estradiol to also test for depression of Vg production at the various wastewater impacted sites compared to the reference site.

Cumulative Effects Monitoring

Session Co-chairs: K.R. Munkittrick and M. Stephenson

Export of Mercury Downstream from Reservoirs. R. Schetagne[1], J.-F. Doyon[2] and J.-J. Fournier[3]. 1Department of Hydraulics and Environment, Hydro-Québec, Montréal, QC; [2]Genivar Group, Québec, QC; [3]Department of Environment, Hydro-Québec, Montréal, QC.

Environmental effects monitoring at the La Grande hydroelectric complex (Québec, Canada) revealed important increases in Hg levels in fish caught downstream from reservoirs. A study was carried out in 1997 immediately below the Caniapiscau Reservoir to identify by which components Hg is transported downstream from reservoirs and to assess the amount of Hg being exported. Stomach contents of lake whitefish (*Coregonus clupeaformis*) captured immediately below the Caniapiscau Reservoir were examined to determine which components transfer Hg from lower trophic levels to fish. The analyses of water samples and drifting organisms collected below the reservoir indicate that the dissolved fraction (<0.45 µm) and the suspended particulate matter (0.45-50 µm) are the major components by which methylmercury is transferred downstream of reservoirs, accounting for 64 and 33%, respectively, of the total amounts exported. Drifting organisms such as plant debris, benthic invertebrates, fish, phytoplankton and zooplankton are much less important pathways for Hg export because of their very low biomass per water volume coming out of the generating station, as opposed to the high biomass of suspended particulate matter. However, zooplankton is the major component by which methylmercury is directly transferred to non-piscivorous fish downstream.

Chronic Toxicity to Medaka of Sediment Retene. Y. Kiparissis, J. Reynolds, N. Henry, C. Ho and

P.V. Hodson. School of Environmental Studies, Queen's University, Kingston, ON.

Retene (7-isopropyl-1-methylphenanthrene) occurs at concentrations up to 2400 mg/kg dw in sediments downstream of pulp mills processing softwood. We measured chronic toxicity of sediment retene to the normal somatic and gonadal development of Japanese medaka (*Oryzias latipes*). Fertilized embryos or newly hatched larvae were exposed to retene-spiked sediments (0.1, 1 and 10 g/kg dry weight) for three months under static conditions (media renewed weekly). Hatching success of embryos exposed to 10 g/kg dw decreased substantially (<50%), and all hatched medaka from the same treatment died after one month of exposure. Mortality was not significantly different from controls in all other treatments. Growth and swimming performance were impaired in medaka exposed as embryos to 1 g/kg dw or exposed as larvae to 10 g/kg dw. Similar responses were observed in a parallel experiment with newly hatched medaka exposed to waterborne retene (5, 50 and 500 µg/L), validating the results of the sediment assays. Histological changes in liver and gonad and expression of secondary sex characteristics are currently being evaluated to determine if retene impairs sexual maturation. These preliminary results indicate that retene is readily bioavailable to fish from sediment, that toxicity occurs within the range of observed environmental concentrations, and that responses were more intense when exposure began at the embryonic stage compared to the larval stage.

Does PAH Potency for CYPIA Induction and AhR-Binding Predict Toxicity to Larval Fish? S.M. Billiard[1], M.E. Hahn[2], J.J. Stegeman[2], R.E. Peterson[3], N.C. Bols[4] and P.V. Hodson[1]. [1]Queen's University, Kingston, ON; [2]Wood's Hole Oceanographic Institute, Wood's Hole, MA; [3]University of Wisconsin, Madison, WI; [4]University of Waterloo, Waterloo, ON.

The potency of PAH for EROD induction may not predict potency for blue sac disease in larval rainbow trout. This contrasts with chlorinated dioxins and PCBs where the rank order for larval toxicity correlates to rank order for CYPIA potency and binding to the AhR. Only retene, the weakest inducer tested, and phenanthrene, a non-inducer, caused blue sac symptoms in larvae. However, exposure-response curves for EROD activity in larvae exposed to PAH were not as clear as curves for a rainbow trout cell line (RTL-W1) or juvenile trout. Therefore we compared PAH concentration-response curves between EROD activity (CYPIA enzyme assay) and immuno-histochemical staining (antibody for the CYP1A protein) to determine: [1] the more sensitive index of induction in sac fry ; and [2] if relative potencies for MFO induction *in vitro* and in juvenile trout are applicable to larval stages. PAH potency for toxicity was also examined in relation to binding affinities for cloned rainbow trout AhR (rtAhR) in competitive binding assays. Preliminary data suggest that PAH tested can bind to trout receptors at concentrations tested (10^{-5} -10^{-6} M). Receptor binding *in vitro* will be compared to whole cell binding affinity and EROD activity in a trout cell line.

Effects of Pesticides on Adrenal Function in Rainbow Trout (*Oncorhynchus mykiss*): Assessment *in vitro* and *in vivo*. M. Bisson and A. Hontela. Université du Québec à Montréal, TOXEN Research Center, Montréal, QC.

Bioasssays for detection and quantitative assessment of chemicals with the capacity to disrupt adrenal steroidogenesis have been developed in our laboratory. Enzymatically dispersed adrenocortical cells of rainbow trout (*Onchorhynchus mykiss*) were exposed *in vitro* to Atrazine, Diazinon, Endosulfan and Mancozeb, and cortisol secretion in response to ACTH (adrenocorticotropic hormone) or dbcAMP (intracellular second messenger), as well as cell viability were determined. The effective dose, EC_{50} (dose that inhibits cortisol secretion by 50%), the median lethal dose, LC_{50} (dose that kills 50% of the cells) and the LC_{50}/EC_{50} ratio were established to assess endocrine toxicity of pesticides evaluated in this study. The pesticides were ranked as follows: EC_{50}, Endosulfan <Diazinon <Mancozeb <Atrazine; LC_{50}, Diazinon <Endosulfan <Mancozeb <Atrazine. For the *in vivo* assessment, fish were exposed

orally (gelatin capsule) to endosulfan for 30 d and various indicators of the physiological stress response were evaluated (cortisol secretion, plasma glucose, liver glycogen). Exposure and dose dependant effects of the pesticides were observed. Our results suggest that the bioassays can be used for screening of adrenotoxicants, for mechanistic studies of adrenotoxicity and for assessment of the impact of pesticides on fish health. (Funded by CNTC and Toxen Research Center).

Mesocosm Study Designs for Canada's EEM Program. N.E. Glozier[1], J.M. Culp[1], K. Cash[1], D.L. MacLatchy[2], M.G. Dube[1], J. Mollison[1], D. Halliwell[1] and C. Casey[1]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Department of Biology, University of New Brunswick, Saint John, NB.

Canada's EEM program has several study design options for conducting fish or invertebrate field surveys. If there are confounding factors which limit the applicability of field programs, one of the recommended alternative approaches is on-site community bioassays conducted with mesocosms. Properly designed mesocosm studies can eliminate these confounding factors by manipulation of aquatic communities under environmentally relevant field conditions and controlled effluent exposures. Recently we designed and constructed a modular mesocosm system based on our previous mesocosm studies. We tested these modular units during separate trials at pulp mill and metal mine sites in New Brunswick as part of a TSRI study. The design modifications allowed increased replication, a system for more remote locations and a reduction in the time frame required for multi-trophic level (i.e., algal, invertebrates and fish) bioassays. We discuss the potential use of the new modular-unit system for addressing the tiered objectives of the MM EEM program and provide some examples of mesocosm study designs and protocols in relation to situations with confounding factors. For example, the cumulative effect of several effluents which are discharged into the same receiving water can be determined using a 4 module system. We conclude that these modifications and study designs can address the EEM objectives while being cost-effective.

Environmental Effects Monitoring in the Ottawa River for Region of Ottawa-Carleton. C.D. Wren[1], M. Trudeau[2], K. Cover[2] and B. Muncaster[3]. [1]ESG International, Guelph, ON; [2]Region of Ottawa Carleton, R.O. Pickard Environmental Centre, Ottawa, ON; [3]ESG International, Ottawa, ON.

A study was undertaken to evaluate the potential effects of facilities operated by the Region of Ottawa Carleton (Region) on the Ottawa River. The Region operates two water purification plants (WPPs) and a large sewage treatment plant (STP). Other discharges into the Ottawa River include over 100 sewers and combined sewer outfalls, more than 14 tributaries near the city that carry urban and agricultural runoff, as well as pulp and paper complexes and other municipal discharges on the Quebec side of the river. Effluent flow from the STP is approximately 5.3 m/s, compared with average flows of >600 m/s in the Ottawa River. The 1% effluent plume extends approximately 400 m downstream of the discharge diffuser. Treated effluent from the STP was consistently non lethal to rainbow trout and *Daphnia magna*, but the growth of juvenile fathead minnows was reduced in sublethal tests. Samples of raw sewage to simulate a by-pass event were consistently lethal to rainbow trout, but were not lethal to *D. magna*. Aluminum hydroxide is used as a flocculating agent in the WPPs to provide clean drinking water. Process water is discharged periodically into the Ottawa River. Sediment toxicity tests showed that sludge from the WPPs resulted in 100% mortality to midge larvae (*Chironomus riparius*), and elicited some effects on survival and growth of the amphipod *Hyallela azteca*. The benthic invertebrate community was clearly impacted downstream of one of the WPPs. The existing monitoring programs of the Region were reviewed, and an integrated environmental effects monitoring (EEM) program was developed for the region. The EEM program is one component of the Region's watershed management approach for municipal systems design and operation.

Agricultural Influences on Fish Ecology and Performance in New Brunswick. M.A. Gray and K.R. Munkittrick. Department of Biology, University of New Brunswick, Fredericton, NB.

One of the major agricultural sectors in North-eastern New Brunswick is potato farming. Potato farming is the most intrusive form of cultivation activity; [1] clearing lands increases the potential for erosion; [2] it requires the relatively intensive applications of chemicals for the control of pests and fungal growths; and [3] it strips the soil of nutrients, increasing the need for fertilizers to be applied. These three factors, soil, chemicals, and nutrients, are all potential agricultural inputs to adjacent streams and rivers during rainfall events. We chose to study the slimy sculpin (*Cottus cognatus*) to assess the potential aquatic impacts of these inputs. This small, relatively sedentary fish species exhibits many desired characteristics for use as an indicator species. Population surveys of slimy sculpin in agricultural reaches show differences in size distributions, fish condition, and organ size. Collections are examining size and age distribution, growth, and reproductive performance in an attempt to understand the relative roles that habitat damage, nutrient enrichment and chemical use play in the responses of the fish. Habitat assessments, water quality and water chemistry were quantitatively evaluated in order to identify associations between impacts on fish population dynamics and agricultural inputs.

What do Early Life Stages of Estuarine Killifish (*Fundulus heteroclitus*) Tell Us About Marine Environmental Quality? S.C. Courtenay[1], L. Van Guelpen[2] and M. Boudreau[1]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]Huntsman Marine Science Centre, St. Andrews, NB.

Estuaries are critical spawning and rearing habitat for many species of fish. However, because these areas provide access to both fresh water and the ocean estuaries also tend to be sites of human settlement and industry. Inevitably as human populations increase in size their impacts on estuaries and estuarine biota increase. This study addresses the need for better tools for measuring marine environmental quality (MEQ) in estuaries. The mummichog (*Fundulus heteroclitus*) was selected as a sentinel species because it is believed to move very little within or between seasons and should therefore reflect local conditions. Early life stages were examined because they are considered to be particularly sensitive to environmental insult. Morphological abnormalities were quantified in larvae and juveniles sampled from areas receiving industrial (pulp and paper) and municipal (treated sewage) effluents in the Miramichi River estuary and Pictou Harbour area and compared to fish from less impacted areas. In addition, laboratory experiments were carried out in which embryos were reared in dilutions of effluents from the pulp mills located on the Miramichi estuary and Pictou Harbour. Endpoints measured included survival, time and size at hatch, and survival and growth of larvae. Preliminary results of these analyses and the potential of this approach for measuring MEQ will be discussed.

Development of a Cumulative Effects Strategy for the Freshwater Portion of the Saint John River. K.R. Munkittrick[1,2], S. Currie[2], M.A. Gray[2], B. Galloway[2] and A. Curry[2]. [1]Environment Canada, National Water Research Institute, Environment Canada, Burlington, ON; [2]Department of Biology, University of New Brunswick, Fredericton, NB.

We have been developing a strategy for dealing with cumulative effects assessment, which uses the performance of resident fish to help understand the level of stress on a river reach. This study is examining the performance of slimy sculpin, white sucker and yellow perch in the upper Saint John River basin to develop a strategy for increasing the level of understanding of the system. The data will be used to give a relative indication of performance within the system and to prioritize areas of concern within the river basin. The first year of the project (1999) collected data from more than 30

sites in the upper river basin. Slimy sculpin were effective at discriminating impacts of closely related discharges on the Saint John River. Agricultural practices were associated with an increased growth rate of sculpin, and a decrease in the abundance of young-of-the-year. The second year of the project (2000) documented fish community structure throughout the freshwater portion of the basin, and expanded the information base on tributary drainages. The final analyses will evaluate the relative strengths of fish and benthic community data, population estimates and fish performance evaluations, for determining the health of the system. Surveys will be conducted on resident fish, habitat suitability and fish and invertebrate communities, to develop a baseline of performance for interpreting other studies. There are more difficulties in dealing with estuarine and marine areas, and preliminary work will enable eventual expansion of the framework to downstream areas.

Incorporating an Effects-Based Approach into a Cumulative Effects Assessment Framework for Aquatic Ecosystems. M.G. Dubé[1] and K.R. Munkittrick[2,3]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Department of Biology, University of New Brunswick, Fredericton, NB.

The cumulative effects assessment (CEA) component of an environmental assessment, which is required under the *Canadian Environmental Assessment Act*, is intended to assess or predict any potential effects of a proposed project relative to the existing "accumulated state" of the environment. Ideally then, CEA would involve two components, an evaluation of the existing environmental state or the existing cumulative response of the environment to existing stressors and, development of a predictive model to determine any potential impacts of additional stressors. The objective of this paper was to propose a more holistic, systematic framework for assessing cumulative effects of existing and proposed project activities on aquatic ecosystems. Framework components includes: [1] an effects-based assessment congruent with the design of the Canadian EEM program to determine the existing accumulated environmental state prior to predicting the impacts of new development; [2] stressor-based assessment congruent with existing environmental assessment practices to predict potential impacts of new development relative to the existing environmental state; [3] incorporation of post-development or follow-up monitoring to assess the accuracy of impact predictions, study design, and to provide an avenue for adaptive management; and [4] incorporation of decision-making frameworks at each informational stage of the process to link scientific information to management decisions and action. Advantages of the proposed CEA framework include building consistency in the CEA process, integration of regional assessment information into a project-specific environmental assessment, links environmental assessment to other forms of practiced environmental management, acknowledges that predictions of development impacts may not be accurate and provides an avenue for adaptive management, and provides quantification of ecologically relevant thresholds for environmental stress.

An Integrated Approach to Cumulative Environmental Effects Assessment, Meeting the Requirements of the ***Canadian Environmental Assessment Act.*** J.L. Barnes, M. Stephenson, and L.H. Davey. Jacques Whitford Environment Ltd., Fredericton, NB. E3B 5C2

ABSTRACT

To meet the requirements of the *Canadian Environmental Assessment Act* (CEAA), proponents must assess the cumulative environmental effects of a project that are likely to result in combination with other projects and activities that have been or will be carried out. The authors review the legal requirements and guidance documentation for cumulative effects assessment under CEAA, and describe an integrated cumulative environmental effects methodological framework that meets these requirements. Examples of application of the methodology to the assessment of aquatic ecosystems and/or components thereof are provided to illustrate the method.

INTRODUCTION

The *Canadian Environmental Assessment Act* (CEAA) came into effect in 1995. One of the key features of the legislation was the requirement for the assessment of cumulative environmental effects. Subsection 16(1) of the Act states that every environmental assessment under the act must consider, among other things, the following factors: [1] the environmental effects of the project, including the environmental effects of malfunctions or accidents that may occur in connection with the project and any cumulative environmental effects that are likely to result from the project in combination with other projects or activities that have been or will be carried out; an [2] the significance of the effects referred to in paragraph (a).

Prior to the coming into force of CEAA, the Canadian Environmental Assessment Agency ("CEA Agency") issued Addressing Cumulative Environmental Effects, A Reference Guide for the *Canadian Environmental Assessment Act* (the "Reference Guide") (CEA Agency 1994a). More recently the Agency published an operational policy statement Addressing Cumulative Environmental Effects under the *Canadian Environmental Assessment Act* (the "Operational Policy") (CEA Agency 1999a). Coupled with the Cumulative Effects Assessment Practitioners Guide (the "Practitioners Guide") (CEA Agency 1999b) that was issued at the same time, the Agency is offering the practitioner guidance on how to assess cumulative environmental effects in the context of the Act. At the same time, the Practitioners Guide is a "best practices guide". It is intended to be broadly applicable across Canadian jurisdictions and was not developed solely in reference to the assessment of cumulative environmental effects under the Act. Notwithstanding this stated intent, the Practitioners Guide is largely silent on the socio-cultural environmental effects that must be considered under CEAA so in that regard it falls short of its intention as a "best practices guide." In spite of this, it offers a broad range of advice on how to assess biophysical cumulative environmental effects and represents an important contribution to the literature on the subject.

Practitioners working to meet the requirements of CEAA must be aware of several important considerations. Firstly, the guidance material of the Agency can be viewed by stakeholders, the public and the Courts as the way in which cumulative environmental effects assessment should be conducted in Canada. The Reference Guide, although an earlier contribution remains fully in effect as official guidance of the Agency. The Practitioners Guide is a supplement and companion to the Reference Guide and does not supersede it. Importantly, the Practitioners Guide is not prescriptive, but rather offers suggestions on how cumulative environmental effects assessments are being or could be done. Lastly, the Practitioners Guide should not be used without consulting the Operational Policy that highlights certain differences between the Practitioners Guide, the Act and the Reference Guide. These considerations are but a reflection of the complexity and lack of certainty that faces the practitioner in assessing cumulative environmental effects.

The Reference Guide identifies five important considerations for the assessment of cumulative environmental effects as described below. Only environmental effects as defined in the Act can be considered cumulatively. Subsection 2(1) of the Act defines environment as: the components of the Earth, and includes land, water and air, including all layers of the atmosphere, all organic and inorganic matter and living organisms, and the interacting natural systems that include components referred to in paragraphs (a) and (b).

An environmental effect under CEAA means, in respect of a project: [1] any change that the project may cause in the environment, including any effect of any such change on health and socio-economic conditions, on physical and cultural heritage, on the current use of lands and resources for traditional purposes by aboriginal persons, or on any structure, site or thing that is of historical, archaeological, paleontological or architectural significance; and [2] any change to the project that may be caused by the environment. Thus, the assessment of cumulative environmental effects must consider: [1]

changes in the environment caused by the project; [2] the environmental effects of any such changes on health and socio-economic conditions, physical and cultural heritage, [3] current use of lands and resources for traditional purposes by aboriginal persons, or any structure, site, or thing that is of historical, archaeological, paleontological, or architectural significance; and [4] any change to the project that may be caused by the environment.

Environmental assessments must consider the cumulative environmental effects that are likely to result from the project in combination with other projects or activities. As noted below, what constitutes "likely" cumulative environmental effects has been the subject of some more recent clarification. The Act states that to be assessed, the effects must result, at least in part, from the project, and only those environmental effects of other projects and activities that accumulate or interact with the environmental effects of the project in question should be included in the assessment.

As a minimum, in addition to past and present projects, the environmental effects of those projects and activities that will be carried out must be considered. The Reference Guide states that the environmental effects of uncertain or hypothetical projects or activities need not be considered. The Reference Guide suggests that it would be prudent to consider projects or activities that are in a government-approvals process as well. The Reference Guide also notes that where projects or activities are not subject to a formal government approvals process but are relevant to the assessment, they should also be considered if there is a high level of certainty that they will occur (e.g., ploughing fields in an agricultural area). The Operational Policy notes that the Practitioners Guide indicates that the selection of future actions to consider in the cumulative environmental effects assessment should reflect the most likely future scenario. While emphasis is given to projects with greater certainty of occurring, hypothetical projects might be discussed on a conceptual basis in some cases. Recognizing the ambiguity between the Reference and Practitioners Guides, the Operation Policy states that responsible authorities should consider projects that are "certain" and "reasonably foreseeable", as recommended by the Practitioners Guide. Reasonably foreseeable projects are defined as those where an action or project may proceed, but there is some uncertainty about this conclusion.

All environmental assessments must consider the significance of the environmental effects of a project, including the cumulative environmental effects. The Canadian Environmental Assessment Agency has published another guide entitled Determining Whether a Project is Likely to Cause Significant Adverse Environmental Effects, A Reference Guide for the *Canadian Environmental Assessment Act* (CEA Agency 1994b). While much of the debate regarding the requirements of the Act on this subject has focused on which future projects are likely, the assessment of past and present projects also present challenges to the practitioner. CEAA puts no limitations on the extent to which environmental effects, including cumulative environmental effects, have to be assessed. Plainly, the Act states that all environmental effects must be assessed without any specific time limitations. The Practitioners Guide offers guidance on how to decide how far back in time to go in the consideration of cumulative environmental effects. In assessing the cumulative effects of a proposed project on fish and fish habitat, for example, do we consider projects that have occurred in the past 5, 10, 20, 50 or 200 years? What are the limits of how far back? Do we have to evaluate the effects of deforestation by early European settlers and the cumulative effects of agricultural and urban development? This paper does not attempt to resolve these questions. We do note however that the responsible authority has some considerable discretion to limit the scope of the project that is being assessed (Section 15(1) of CEAA) and for the "scoped project", to determine the scope of the factors to be considered (Section 16(3) of CEAA). As such, the onus is on responsible authorities to define how far back and which projects should be assessed. Similarly, the scope of the assessment of factors can be limited. This subject has been debated in several Court proceedings relating to the adequacy of assessment conducted under CEAA and must be a constant consideration for the practitioner.

Practical Methodology for The Integrated Assessment of Cumulative Environmental Effects

Over the past decade, the authors' firm has undertaken numerous environmental assessments under various jurisdictions in Canada and internationally, including under CEAA. Many of these environmental assessments, particularly for those projects that were sensitive or larger scale, were integrated cumulative environmental effects assessments. We have had to adapt our evolving methodology in only minor ways to address the requirements of CEAA.

Since the advent of the Act and its associated guidance documentation, we have focused on a clearer articulation and documentation of the consideration of cumulative environmental effects, particularly those that would be classified as "likely future." Unquestionably, before CEAA, any good environmental assessment considered cumulative environmental effects at least to some extent. Indeed, cumulative environmental effects are hard to ignore. For example, a project with discernible environmental effects on an endangered species cannot overlook the circumstances that lead to its current status, i.e., those past and present cumulative environmental effects.

The Reference Guide observes that there are two possible ways to conduct cumulative environmental effects assessment: [1] as a separate section summarizing the methodological approach taken and the result of the analysis; and [2] as an integral part of the analysis. It is our view that the former suggestion has some serious limitations related to the difficulty of treating cumulative environmental effects independently from those direct and indirect environmental effects of the project. There has been a tendency by some practitioners to adopt the former method because of a desire to make sure that interested parties can clearly see that the cumulative environmental effects assessment has done. However, the separation of cumulative environmental effects from the environmental effects of the project is an artificial exercise that does not reflect the way in which environmental effects are manifested. Hence, our only conclusion is that cumulative environmental effects assessment should be integral to any good environmental assessment.

Our environmental assessment methodology under CEAA addresses the scope of the project as defined in Subsection 15(1) of the Act and the factors to be considered as identified under Section 16. We achieve this by following these seven basic steps to assess environmental effects, including cumulative environmental effects: [1] identify issues through scoping and select Valued Environmental Components (VECs) to focus the environmental assessment; [2] istablish boundaries for the environmental assessment and residual environmental effects rating criteria for determining the significance of the environmental effects for each VEC; [3] identify those past, present and likely future projects that could result in environmental effects in combination with the project; [4] identify environmental effects of project activities, by project phase, including those resulting from the interaction of the project with the environmental effects identified for past, present and likely future projects; [5] evaluate the environmental effects, including cumulative environmental effects, using the significance criteria identified in Agency guidance documentation (CEAA 1994b) in light of proposed mitigation; [6] analyze the environmental effects and predict their significance by applying the residual environmental effects rating criteria; and [7] outline a monitoring and follow-up program.

The following describes these steps. A hypothetical project, the construction of an access road to a radio transmitter station in a partially forested rural agrarian part of Canada, is used to help illustrate our approach.

Step 1 - Issues scoping and the selection of Valued Environmental Components.

Ideally, scoping involves consultation with all stakeholders, including the general public, and the application of professional judgement. Our objective during the issues scoping process is to identify the best way to organize or "package" issues into VECs that make sense for the focused analysis of potential environmental effects. For the biological and physical environment, VECs may represent "key" or "indicator" species, communities, species groups, or ecosystems, as well as, "pathways" (e.g.,

air, water), which act as media for the transfer of environmental effects. VECs may also reflect issues that are socially, culturally or economically of value. In our hypothetical example of a transmitter station, because of the development of an access road with a culvert crossing of fish habitat, we might select Atlantic salmon as a key indicator species of aquatic ecosystem health and also because of its cultural and economic value on a local and regional scale. Alternately, we might have chosen from a range of other potential VECs. "Aquatic ecosystems" might have been chosen if there was no specific species of economic or social importance, but rather, the ecosystem as a whole was valued from a scientific perspective. Often, practitioners choose "fish and fish habitat" as a VEC to reflect the component of the ecosystem that is valued, while not necessarily singling out a particular species or species group. In the latter instance, where several salmonid species could potentially be important, "salmonids" could be chosen as the VEC. In other instances, "water quality", a pathway VEC, could be selected as a surrogate for other biophysical components. The ultimate decision on what should be the VEC or VECs must reflect an informed understanding of the potential project-environment interactions, the importance of components to ecological integrity, their sensitivity to the planned perturbations, and the values of society. Regardless, practitioners must use their good professional judgement in consideration of all or many of these factors, including the opinions expressed to them by the various participants in the scoping process.

Step 2 - Establishment of boundaries and residual environmental effects rating criteria.
Boundaries

An important aspect of the environmental assessment process is the determination of boundaries because they focus the scope of work, allowing for a meaningful analysis of potential environmental effects associated with a project. They also aid in determining the most effective use of available study resources. There are two distinct types: temporal and spatial boundaries of the project and the VEC; and administrative and technical boundaries of the assessment.

The first type of boundary is defined by the temporal and spatial characteristics of the project and various VECs. For example, ecological, socio-cultural, economic, health, heritage, traditional land use, and project boundaries are of this type. These boundaries encompass those periods and areas during, and within which, the VECs are likely to interact with, or be influenced by, the project. These boundaries may extend well beyond site limits, even the limits of potential direct interactions between the project and the VECs, particularly in the case of migratory species, or regional or national socio-cultural and economic systems.

Returning to our hypothetical example, in setting the temporal and spatial boundary for Atlantic salmon, we must be careful not to choose an arbitrary assessment boundary. For example, we cannot limit the boundaries to the project zone of influence (a project boundary) since these species of migratory fish may spend part of their life at sea or upstream. Therefore, one needs to set the assessment boundary to be consistent with a relevant population/stock. An important question is whether we are dealing with a genetically distinct, culturally important or commercially important population as often may be the case for Atlantic salmon (although obviously less so now from a commercial perspective). In such instances, the VEC boundary refers to a specific stock from the affected watershed, as opposed to the Atlantic salmon of the Northwest Atlantic Ocean or some other broad regional aggregation. Here, consideration of such potential environmental effects such as the loss of genetic bio-diversity may cause the practitioner to set very narrow and specific boundaries. Understanding the life cycle of the stock and species in question is fundamental (e.g., migratory, anadromous, high fidelity for native stream). For temporal boundaries, knowing the timing of migratory movements and habitat use is also critical.

The second type of boundary addresses the limitations on the scope of, or approach to, work possible during the assessment of environmental effects. These boundaries are referred to as administrative boundaries and technical boundaries to the assessment, and are imposed by such factors as finite

resources of data, time, cost, and labour, as well as technical, political, or administrative reasons or jurisdictions.

Administrative boundaries refer to the temporal and spatial dimensions imposed on the environmental assessment for political, socio-cultural, and economic reasons. Again, returning to our hypothetical example, spatial administrative boundaries could include such elements as fisheries management zones. Temporal administrative boundaries can reflect such considerations as the time available for conducting and reviewing the environmental assessment.

Technical boundaries represent the technical limitations on the ability to evaluate or predict potential environmental effects of the project. For example, it may be difficult to measure or predict the number of individuals of any particular species that might be affected by a project. For Atlantic salmon, population estimates may have wide error limits and the stock will likely be subject to wide inter-annual variations due to a whole range of natural and anthropogenic factors (the latter of which may indeed be the manifestation of cumulative environmental effects). Where such technical boundaries exist, it is important that they be acknowledged.

Residual Environmental Effects Rating Criteria

Fundamental to our approach is the determination of significance. Under CEAA, the determination of significance is central to decision-making. In this regard, we have embraced the guidance documentation of the Agency (CEA Agency 1994b). It has been our experience that this guidance provides us with a sound basis for the determination of significance. We find it flexible, working across the full range of biophysical and socio-cultural and economic VECs.

Rating criteria are specifically defined for each VEC to provide the threshold for determining the significance of residual environmental effects. Residual environmental effects rating criteria are established based on information obtained in issues scoping, available information on the status and characteristics of the VEC, and professional judgement. We normally apply rating criteria recommended by the Agency (CEA Agency 1994b). These rating criteria determine at which points (thresholds) the VEC would experience environmental effects of sufficient geographic extent, magnitude, duration, frequency and/or reversibility to affect its integrity (each of these are described in more detail in STEP 5). These evaluation criteria for determining significance help to frame a rating criterion for significance that reflects a VECs sensitivity to perturbation and its ability recover.

In developing rating criteria for residual environmental effects, one first needs to define which population, stock, community, or ecosystem is represented by the VEC, or in the case of abiotic biophysical components like air or water quality, which airshed(s) or watershed(s), respectively, is affected. For socio-cultural and economic VECs, one must determine what persons, groups of people or communities are affected.

Returning to our example, in developing a residual environmental effects rating criterion for Atlantic salmon, we must determine whether we are considering a stock in a watershed, all the Atlantic salmon in a specific region or all Atlantic salmon. Once our population is defined, we would relate our criterion to appropriate scales of magnitude, frequency, duration, and reversibility of the environmental effect and the overall ecological context. A resulting residual environmental effects rating criterion for Atlantic salmon, encompassing the aforementioned evaluation criteria for determining significance, could be defined as follows. A significant environmental effect is one that affects the population of Atlantic salmon in the watershed in question, in sufficient magnitude, or of sufficient duration and frequency, to cause a decline in abundance and/or change in recruitment (reproduction and immigration from unaffected areas) that would not return that population to its former level within several generations. This example of a residual environmental effects rating criterion is based on the notion of population-based criteria introduced by Conover *et al.* (1985) and as described by Barnes and Westworth (1994)

to also include socio-economic VECs. The authors' firm has applied this approach in numerous assessments for a wide range of biological, physical, socio-cultural and economic VECs. The challenge remains that some considerable degree of professional judgement is needed to evaluate whether the predicted environmental effects, including cumulative environmental effects (e.g., loss of habitat, mortality, lack of reproductive success, loss of genetic bio-diversity), will exceed the designated threshold of significance. It is our experience in most cases that the significance is obvious when compared to the criteria in light of the various data and information contained in the analysis. However, in some instances, lack of previous experience, insufficient data, or the use of predictive tools may cast sufficient uncertainty that it may be difficult to apply the criteria with a high degree of certainty. This is a technical limitation or boundary of the environmental assessment. A precautionary approach to mitigation or the crafting of significance criteria that incorporate some appropriate margin of safety to compensate for the level of uncertainty can assist in dealing with this potential problem.

Step 3 - Identification of past, present and likely future projects.

As mentioned previously, a crucial component of assessing cumulative environmental effects under CEAA includes the identification of past, present and likely future projects that could interact in combination with the project. These are preferably identified during the scoping stage of the environmental assessment. Information on such projects can be obtained from local, municipal, regional, and where appropriate, national and international governments and agencies that may be involved in the approvals process. A formalized request should be made to the regulatory agencies to ensure that all past, present and likely future projects are identified and assessed. Other on-going projects or activities such as traditional uses of land, which may not be subject to permit or approval,

Table 1. Project-Environmental Effects Interaction Matrix
Valued Environmental Component: Atlantic Salmon

Project Activities and Physical Works	Habitat loss	Habitat fragmentation	Direct mortality	Loss of genetic biodiversity
Construction				
Clearing	X			X
Stripping	X			X
Cutting and Filling	X			X
Stream Crossings	X	X	X	X
Quarrying	X			X
Operation				
Project Activity 1,	etc.			
Project Activity 2, etc.				
Decommissioning				
Project Activity 1,				
Project Activity 2, etc.				
Malfunctions, Accidents and Unplanned Events				
Project Activity 1				
Project Activity 2, etc.				
Past, Present, and Likely Future Projects				
Agricultural Land Use in Watershed	X	X	X	X
Forest Resource Harvesting in Watershed	X	X	X	X
Lucky Strike Mine	X	X	X	X
Electrical Power Transmission Line Construction	X	X		X
Trans Canada Highway and Route 10	X	X		X
Aboriginal Subsistence Fishery			X	X
Recreational Fishery			X	X

must also be identified. Once identified, these projects or activities are listed in tables, which include relevant information such as location and, in the case of likely future projects, their status.

Past and present projects also help to establish the existing conditions or status for each VEC. The description is restricted to a discussion of the status and characteristics of the VEC within the assessment boundaries established for it. The discussion of existing conditions is based on available data, field reconnaissance, and to a large extent, professional experience and judgement. For Atlantic salmon, it might entail characterization of habitat that might be affected by the project. In such instances, evaluation of the condition of the habitat (e.g., habitat mapping, water and sediment quality assessment, benthos, presence of forage species) may reflect the aggregated cumulative environmental effects of other past and present projects (e.g., forestry, agriculture, mining, subsistence harvesting). Other analyses might include population and migration studies, and tissue analysis for contaminants and body burdens of metals. Decisions on how to characterize the existing environment must reflect the nature of past and present environmental effects and those that may result from the proposed project and likely future projects.

It is also important to give consideration to the likely future conditions of the receiving environment without the project. These likely future conditions are based on known trends, past, present and planned future projects and activities, and professional judgement. Any data gaps regarding both existing and likely future conditions should be identified, including a discussion of how such gaps influence the analysis.

Step 4 - Identification of project environmental effects.

This step involves the identification of VEC-specific project related environmental effects (i.e., project-VEC interactions) and a description of issues and concerns regarding key interactions. In order to standardize this step and in keeping with standard practice, the authors' firm has developed a project-environmental effects interaction matrix template (Table 1) specifically for each VEC. It describes the scope of the environmental assessment for each VEC and is limited to only those interactions identified through scoping. It also identifies "other-project" activities (i.e., past, present and likely future projects that act in combination with the project) that could result in potential environmental effects that act cumulatively with the project.

Returning to our example of the Atlantic salmon VEC, project activities during the construction phase could include clearing, stripping, cutting and filling, stream crossings, and quarrying for access road development and site preparation. These activities could potentially result in environmental effects such as habitat loss, habitat fragmentation, direct mortality, and loss of genetic bio-diversity. These interactions are shown in Table 1 as an illustration. Beyond the construction phase example, the interactions for all project phases would be assessed as well as the identified past, present and likely future projects identified for the evaluation of cumulative environmental effects. In addition to listing of the potential project-environmental effects interactions in Table 1, key issues and concerns arising from these interactions would be highlighted in the accompanying text of the environmental effects analysis.

Step 5 – Evaluation of environmental effects.

The next step in our assessment process consists of the evaluation of potential residual environmental effects, by project phase, in light of proposed specific mitigation and the evaluation criteria for determining significance described by the Agency (CEAA 1994b). The purpose of this step is to evaluate the interactions between project activities and the VECs and to determine the nature and extent of residual environmental effects, i.e., those effects that may persist after all mitigation strategies have been implemented. As most projects involve at least some kind of environmental effect, it has become practice to evaluate the significance of those effects. The significance of environmental effects is determined in step 6, on basis of the evaluation conducted in steps 4 and 5.

Table 2. Environmental Effects Assessment Matrix Template: M (Magnitude); GE (Geographic Extent); D/F (Duration/Frequency); R (Reversibility); E/S (Ecological/Socio-cultural economic context).

Valued Environmental Component: Atlantic Salmon
Phase: Construction

Project Activity	Potential Positive (P) or Adverse (A) Environmental	Mitigation	Evaluation Criteria for Assessing Environmental Effects				
			M	GE	D/F	R	SE
Clearing	Habitat Loss (A)	Erosion control measures Riparian buffer zones	1	1	2/1	R	2
	Loss of genetic bio-diversity (A)	Habitat enhancement Habitat replacement Erosion control	1	1	2/5	R	2
Stream Crossings	Habitat Loss (A)	Habitat enhancement Habitat replacement	1	1	1/1	R	2
	Habitat Fragmentation (A)	Ensure fish passage through design of culverts and bridges	1	1	1/1	R	2
	Direct Motality (A)	Use barrier nets and electro-seining to remove fish from work area prior	1	1	1/1	R	2
	Loss of genetic bio-diversity (A)	Habitat enhancement Habitat replacement	1	1	1/1	R	2
Etc.							

Key:

Magnitude:
1 = Low. e.g., a few individuals affected occasionally over a period of less than two weeks.
2 = Medium. e.g., small portion of the local Aboriginal community affected often, over a period of more than one month.
3 = High: e.g., a large portion of the local Aboriginal community affected continuously over a period of more than one month.

Ecological/Socio-cultural and Economic Context:
1 = Relatively pristine area or area not adversely affected by human activity
2 = Evidence of adverse effects

Geographic extent:
1 = < 1 km^2
2 = 1-10 km^2
3 = 11-100 km^2
4 = 101-1000 km^2
5 = 1001-10,000 km^2
6 = >10,000 km^2

Duration:
1 = <1 month
2 = 1-12 months

N/A = Not Applicable

Frequency:
1 = <11 events/year
2 = 11-50 events/year
3 = 51-100 events/year
4 = 101-200 events/year
5 = >200 events/year
6 = continuous

Reversibility:
R = Reversible
I = Irreversible

The evaluation of environmental effects, including cumulative environmental effects, takes into consideration: [1] the potential interaction between project activities, for each of the project phases, and their environmental effects in combination with those of other past, present and likely future projects (as described in STEP 4); [2] the mitigation strategies applicable to each of the interactions;

and [3] the Agency's evaluation criteria for determining significance (CEAA 1994b).

An environmental effects assessment matrix template (Table 2) is used to summarize the analysis of environmental effects, including cumulative environmental effects, by project phase, including malfunctions, accidents and unplanned events. This allows for a comprehensive analysis of all project-VEC interactions in a matrix format. Supporting discussion in the accompanying text highlights particularly important relationships, data, or assessment analysis, but does not necessarily address all items noted in the table. For illustration, Table 2 shows the construction phase for Atlantic salmon for the example radio transmitter station project. The first two columns describe the project activity and its anticipated environmental effect(s) as provided in Table 1. The third column lists specific mitigation that will be applied for each project-environmental effect interaction. The last grouping of columns rate the environmental effects based on specific evaluation criteria for determining significance (CEAA 1994b). Each are further defined below.

Classifying anticipated environmental effects

The concept of classifying environmental effects simply means determining whether they are adverse or positive. The following includes some of the key factors that are considered for determining adverse environmental effects, as per the Agency guidelines (CEAA 1994b): [1] negative effects on the health of biota; [2] loss of rare or endangered species; [3] reductions in biological diversity; [4] loss or avoidance of critical/productive habitat; [5] fragmentation of habitat or interruption of movement corridors and migration routes; [6] transformation of natural landscapes; [7] discharge of persistent and/or toxic chemicals; [8] toxicity effects on human health; [9] loss of, or detrimental change in, current use of lands and resources for traditional purposes; [10] foreclosure of future resource use or production; and [11] negative effects on human health or well-being.

Mitigation

Mitigation includes environmental design, environmental protection strategies, and mitigation specific to the minimization or control of potential adverse environmental effects of a particular VEC. As required by CEAA, these measures must be technically and economically feasible. In the case of positive effects, enhancement opportunities are considered. For Atlantic salmon, mitigation measures can involve, for example, a suite of environmental protection procedures during construction, replacement of lost habitat, habitat enhancement, removal of barriers to migration and the opening up of previously inaccessible habitat.

Application of evaluation criteria for assessing environmental effects

Several criteria are taken into account when evaluating the nature and extent of environmental effects. These criteria include, as stated previously (CEAA 1994b): Magnitude; Geographic extent; Duration and frequency; Reversibility; and Ecological, socio-cultural, and Economic context. Each criterion has a numeric descriptor in the key of the environmental effects assessment matrix (Table 2) to simplify the presentation of results of the environmental assessment. It should be noted, however, that the template in Table 2 is an example for a specific biological VEC. Other VECs would use, as appropriate, different criteria in the tables' key to reflect the specific characteristics of those VECs.

Step 6 – Analysis and prediction of the significance of environmental effects.

The analysis and prediction of the significance of environmental effects, including cumulative environmental effects, encompasses the following: [1] determination of the significance of residual environmental effects, including cumulative residual environmental effects, for each phase of the project and for the project overall; [2] establishment of the level of confidence for predictions; and [3] determination of scientific certainty and probability of occurrence of the predicted residual environmental effects.

Table 3. Residual Environmental Effects Summary Matrix Template

Valued Environmental Component:

Phase	Residual Environmental Effects Rating, Including Cumulative Environmental Effects*	Level of Confidence	Likelihood	
			Probability of Occurrence	Scientific Certainty
Construction	S	3	3	3
Operations	S	3	3	3
Decommissioning	S	3	3	3
Malfunctions, Accidents and Unplanned Events	NS	1	1	1
Project Overall	S	3	3	3

*As determined in consideration of established residual environmental effects rating criteria.

Key:

Residual environmental Effect Rating
- S = Significant Adverse Environmental Effect
- NS = Non-significant Adverse Environmental Effect
- P = Positive Environmental Effect

Level of Confidence
- 1 = Low Level of Confidence
- 2 = Medium Level of Confidence
- 3 = High Level of Confidence

N/A = Not Applicable

Probability of Occurrence: based on professional judgement
- 1 = Low Probability of Occurrence
- 2 = Medium Probability of Occurrence
- 3 = High Probability of Occurrence

Scientific Certainty: based on scientific information and statistical analysis or professional judgement
- 1 = Low Level of Confidence
- 2 = Medium Level of Confidence
- 3 = High Level of Confidence

Upon completion of the evaluation of environmental effects in STEP 5, the residual environmental effects are assigned an overall rating of significance for each of the project phases (e.g., construction, operation, decommissioning, and malfunctions, accidents, and unplanned events). This overall determination considers all residual environmental effects, including project and other-project cumulative environmental effects. As such, this represents an integrated residual environmental effects evaluation. These are presented in the residual environmental effects summary template (Table 3) for the example project and Atlantic salmon. This table provides a phase-by-phase and a project overall residual environmental effects rating. Where significant adverse or positive residual environmental effects are predicted, a level of confidence and likelihood of occurrence rating are also given to each prediction. Each are described below.

Significance Rating

Taking into consideration the analyses conducted in STEPS 4 and 5, a phase-by-phase and an overall rating of significant or not-significant is assigned. A rating of positive may also be applied. Specific thresholds for determining significance are developed for a VEC to reflect the distinction between those environmental effects that should or should not be collectively considered to be significant.

The rating of significance is determined by the aggregate consideration of project-related environmental effects and those of other past, present and likely future projects against the thresholds

that have been established for the specific VEC, and within the defined environmental assessment boundaries established for that VEC. Significant environmental effects are those which are considered to be of sufficient magnitude, duration, frequency, geographic extent, and/or reversibility to cause a change in the VEC that will alter its status or integrity beyond an acceptable level. Establishment of the criteria is based on professional judgement, but is transparent. In conducting this judgement, the accompanying text must highlight specifically how the cumulative environmental effects have been factored into the determination of the significance of residual environmental effects.

An inherent problem can arise in this analysis where other projects are the major contributors to cumulative environmental effects. For example, the project related environmental effects on Atlantic salmon may be of a minor nature, but the entire project region is subjected to extensive forestry and agricultural activities that are having far-reaching cumulative environmental effects that far outweigh the contribution of the project. Barnes and Westworth (1994) devised a scheme where the proportion of contribution of the project to overall cumulative environmental effects was enumerated on the basis of professional judgement. Such analysis is of considerable use to decision-makers in evaluating the acceptability of cumulative environmental effects and for the attribution of responsibility for required mitigation and monitoring. In Table 3, we have indicated that the project environmental effects are significant on Atlantic salmon. In this fictional example, it is highly likely that the project related environmental effects are not significant. However, in combination with those other past, present and likely future projects, we may have been forced to acknowledge extensive cumulative environmental effects. Obviously, in this situation, the assessor must transparently attribute the responsibility of these cumulative environmental effects to the other projects.

Responsible authorities under CEAA can do much to minimize this type of methodological problem by carefully exercising their discretion to eliminate past, present and likely future projects that are outside of the responsibility of the proponent or that are permitted activities (e.g., forestry, agriculture, fishing). In this regard, scoping can do much to minimize the complexity and maximize the relevance of the environmental assessment.

Level of confidence

The significance of the residual environmental effects of the project on VECs is evaluated based on a review of relevant literature, consultation with experts, field reconnaissance, and professional judgement. In some instances, making predictions of potential residual environmental effects is difficult due to the limitations of available data (e.g., technical boundaries). Ratings are therefore provided in Table 3 to indicate, qualitatively, the level of confidence for each prediction.

Determining Whether Or Not Significant Environmental Effects Are Likely To Occur.

The likelihood of significant adverse residual environmental effects is important for establishing priorities (e.g., for mitigation) and to guide decision-making in respect of the project. In keeping with Agency guidance documentation (CEAA 1994b), we use the following criteria for the determination of likelihood:

Probability of occurrence; and Scientific certainty.

Where possible, it is desirable to apply rigorous scientific and/or statistical methods to determine the likelihood of significant residual environmental effects. Where such methods are not feasible, it is necessary to use a qualitative approach to determine the likelihood, based on other non-statistical analyses or through professional judgement. Where applicable, an indication of the scientific certainty associated with the determination of likelihood is made based on reference to scientific knowledge, statistical significance, quantitative risk assessment, or professional judgement. A rating is also provided when environmental effects are positive.

Step 7 – Monitoring and follow up

As part of the environmental effects analysis, appropriate monitoring and follow-up are described (i.e., environmental effects monitoring or EEM). In developing an EEM program, the results of Steps 1 through 6 are helpful in focusing on important interactions, where there is a high level of uncertainty about environmental effects predictions, where significant environmental effects are predicted, or in areas of particular sensitivity. As noted earlier, the contribution of the project to overall cumulative environmental effects may influence decisions regarding the responsibility for monitoring.

As in the environmental assessment process, it is important for the EEM practitioner to understand both the scope of the assessment, and the scope of the factors to be assessed. The authors believe that as the consideration and assessment of the cumulative effects of projects have been driven and defined through the CEAA framework, the development of EEM programs stemming from the CEAA process should also reflect this framework. In this respect, the translation of VECs from an environmental assessment into manageable and testable hypotheses for an EEM program has parallels in the Ecological Risk Assessment (ERA) methodology (CCME 1996; U.S. EPA 1996). In ERA, VECs are defined, and assessment endpoints are stated. An assessment endpoint is an explicit expression of the environmental value that is to be protected. For example, Atlantic salmon is a valued ecological entity, and the maintenance of a viable salmon population would be a reasonable assessment endpoint for either an EA, or an ERA. However, although assessment endpoints can be clearly stated and are suitable management objectives, they are generally not clearly enough defined to provide a basis for monitoring programs. Thus, as in the ERA methodology, they need to be translated into measurement endpoints or measures of effect. A measure of effect is a measurable ecological characteristic that is related to the assessment endpoint. In our hypothetical example, measurement endpoints could include the number of spawning Atlantic salmon entering a stream reach, the number of eggs deposited, or the number of parr produced per unit of fish habitat.

CONCLUSIONS

To meet the requirements of CEAA, an integrated cumulative environmental effects assessment framework has been described. The framework involves seven flexible steps that enable the practitioner to systematically evaluate the environmental effects of the project in combination with past, present and likely future projects. The analysis is summarized in template matrices for comprehensiveness, while the accompanying text highlights important elements of the analysis. The authors' firm has used this basic approach for over a decade in a range of jurisdictions. The methodology has been successfully used in numerous environmental assessments and subjected to the intense scrutiny of public hearings by a Review Panel, the National Energy Board and the Federal Court of Canada, all of which have accepted its validity and appropriateness.

The authors believe that the development of EEM programs that take into consideration cumulative effects should give careful consideration to the experience gained in the environmental assessment process. Factors that should be explicitly and carefully considered, and if necessary limited, in order to develop manageable and meaningful EEM programs include the selection of VECs, the scope of the assessment, and the scope of factors to be assessed. Finally, the translation of assessment endpoints into measurement endpoints is also a critical process if scientific rigour is to be maintained.

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Ecological Risk Assessment

Session Co-chairs: C.E. Moore and L. Rutherford

Ecological Risk Assessment of Contaminated Ponds Located at the Former U.S. Naval Base in Argentia, NF. C.E. Moore[1], L.J. Marshall[1], R.D. Willis[1], K. Walker[2] and G. Troke[2]. [1]Cantox Environmental Inc., Halifax, NS; [2]Public Works and Government Services Canada, Placentia, NF.

The former U.S. Naval Base at Argentia, NF was closed by the U.S. Navy in 1994, and has undergone a series of extensive environmental studies since that time to determine the nature and extent of contamination present within the base property, as well as the potential risks this contamination may pose to ecological and human receptors. In 1998, an ecological risk assessment of 5 contaminated ponds was conducted, in order to assess whether the contamination posed a risk to species within the pond, or those using the ponds as foraging areas. This paper will present an overview of the study, focusing on key issues and highlighting approaches taken to assess risk.

Unique Considerations when Conducting an Ecological Risk Assessment for a Wide-Area Site. B. Duncan[1], R.N. Hull[2], G. Brown[2] and R. Sentis[1]. [1]Cominco Ltd., Trail, BC.; [2]Cantox Environmental Inc., Mississauga, ON.

Cominco recently completed a human health risk assessment for a smelter operation which did not indicate the need for extensive remediation; however, an ecological risk assessment remains to be done to develop a complete remediation plan for the area. Airborne releases of chemicals such as

SO_2 and metals (Pb, Zn and others) have resulted in contamination of the valley around the site. This complicates the ERA because: [1] topography influences the distribution of contaminants along the river valley; [2] background concentrations of metals are naturally high, as the area is an historical mining area; [3] other industries located upstream of the site contribute effluent to the river; and [4] there are ongoing emissions from the smelter as well as historical contamination. Because of the size of the area being evaluated, this ERA has been identified as a "wide-area site ERA" under the *Contaminated Sites Regulation* in B.C. This ERA is one of the first such ERAs to be conducted, and as such, particular attention is being given to the problem formulation stage. This stage is detailed, including the identification of habitats, selection of receptors, screening of chemicals of concern, identification of exposure pathways, identification of data gaps, and the initiation of a public consultation process.

Ecotoxicological Risk Assessment of Explosives-Contaminated Sites. P.Y. Robidoux[1], J. Hawari[1], P. Gong[1], S. Thiboutot[2], C. Dubois[2] and G.I. Sunahara[1]. [1]Biotechnology Research Institute, National Research Council of Canada, Montreal, QC; [2]Defense Research Establishment - Valcartier, Canadian Ministry of National Defense, Val Bélair, QC.

Explosives such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) are recalcitrant munition chemicals released into the environment at factory sites, military areas such as firing ranges and open burning-detonation (OB/OD) areas, and through field use. These compounds may be toxic at relatively low concentrations to a number of ecological receptors. A preliminary ecotoxicological risk assessment approach was used for explosive-contaminated sites from a factory and military training area. This presentation summarizes the approach taken and presents recent results of a case study using a TNT-contaminated site. Toxicity data from the literature and laboratory data were compared to the exposure assessment to estimate the risk for ecological receptors. Assessment endpoints considered were direct effects on soil (microbial process, plants and invertebrates), wildlife (mammals and birds) and aquatic (micro-organisms, microphytes, macrophytes, invertebrates and fish) species. Toxicity studies on the earthworms reproduction and field experiments in mesocosms were carried out. A simple biomarker, the neutral red retention time, was used to assess the sub-lethal toxicity to the earthworms. The uncertainty associated at the different ecological risk assessment procedure steps was also considered. In general, the selected areas which were significantly contaminated by explosives such as TNT or HMX may constitute a potential risk of effect on soil organisms and mammals.

Ecological Risk Assessment for a Lower Reach of the Waterford River, St. John's, NF. H.S. Schillereff[1], K. Knight[2], M. Stephenson[3] and D. Pinsent[1]. [1]Jacques Whitford Environment Ltd., St. John's, NF; [2]Public Works and Government Services Canada, Environmental Services, St. John's, NF; [3]Jacques Whitford Environment Ltd., Fredericton, NB.

Using the CCME framework, a Tier 1 Ecological Risk Assessment (ERA) was carried out for the terminal 800 m reach of the Waterford River in an urban industrialized area adjacent to a former rail yard undergoing redevelopment. The ERA was enhanced by assessing vegetation and fish habitat along the river and by analysis of river sediment, river water, benthic community samples and shallow groundwater at five locations along the reach. The variety and distribution of vegetation along the reach shows limited diversity and some barren soil areas (due to road salt in snow dumps). Most of the reach is Type II (spawning and rearing) salmonid fish habitat (moderate riffles, pools, current 0.3-1 m/s, depth 0.2-1.5 m, substrate cobbles/boulders with patchy sand), although river stage increases dramatically (up to 2 m) during spring snow melt runoff. Petroleum hydrocarbon, PAH and metal concentrations of river sediment and water were compared with known and inferred concentrations of

lateral inputs to the reach from groundwater and storm sewer discharges. A chemical mass balance for the reach showed that the mass fluxes from upstream river inflow were five to six times greater than lateral inflows for petroleum hydrocarbons, two times greater for metals and about equal for PAHs. Mass fluxes from groundwater discharge from the rail yard were much less than river inflow and sewer discharge inputs. Sub-chronic survival/growth rates for the amphipod *Hyallela azteca* showed good survivability and growth (similar to control sets) except near a local area of known petroleum hydrocarbon in bank soils (since remediated) and at a large municipal sanitary sewer outfall. Valued Environmental Components (VECs) used for the ERA included brook trout (*Salvelinus fontinalis*), American black duck (*Anas rubripes*) and mink (*Ondatra zibethicus*). Hazard severity and exposure assessment for these VECs were combined to determine qualitative risk levels based on principal exposure pathways of direct uptake/ingestion of sediment and uptake from food chain. Based on ERA results and accounting for very conservative assumptions, the adverse effects to VEC species were considered very unlikely to unlikely. The general environmental health of this reach can be considered fair considering its history and adjacent land use.

Canadian Environmental Quality Guidelines for Nonylphenol and its Ethoxylates. K.L. Potter, K. Adare, L. Trudel and C. Gaudet. Environment Canada, National Guidelines and Standards Office, Hull, QC.

Nonylphenol and its ethoxylates are among the substances on the second Priority Substances List of the *Canadian Environmental Protection Act* (CEPA). These substances have been proposed "toxic", as defined in section 64 (a) of CEPA, because they are entering the environment "in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity." Nonylphenol ethoxylates are nonionic surfactants that are widely used in detergents and in a variety of industrial processes. Although they readily break down upon release to the environment, some of their metabolites, including nonylphenol, are much more persistent and toxic than the parent compounds, and have shown endocrine disrupting effects. *Canadian Environmental Quality Guidelines* (CEQGs) for water, sediment and soil are being developed for these substances based on nationally approved protocols. CEQGs are nationally endorsed, science-based goals that are recommended as levels that should result in negligible risk to biota and ecosystem health. These guidelines serve as benchmarks of environmental quality that can be used nationally for the protection, evaluation, and enhancement of the Canadian environment and its beneficial uses. Guideline development issues that are relevant to nonylphenol and its ethoxylates include the difficulty in dealing with chemical mixtures and the ecological significance of endocrine disrupting effects.

Categorizing and Screening Canada's Domestic Substances List: Identifying PBT Substances in Canadian Commerce. P.W. Harris, M. Berci, R.L. Breton, D.W. Gutzman, D. MacDonald, J. Sanderson and R. Sutcliffe. Environment Canada, Commercial Chemicals Evaluation Branch, Hull, QC.

Recently, the revised *Canadian Environmental Protection Act* (CEPA '99) came into effect, bringing new initiatives for pollution prevention and reducing toxic substances in the environment. One of the initiatives currently underway involves the identification of substances on the Domestic Substances List (DSL) that are persistent or bioaccumulative, and toxic (PBT). The DSL currently contains approximately 23,000 substances and includes organic chemicals, inorganic substances, polymers, and substances that are of unknown or variable composition. If a given substance is determined to be persistent or bioaccumulative, and toxic to environmental biota based on categorization criteria, then that substance will proceed to a screening level risk assessment to determine if it poses a risk to the Canadian environment. This presentation will provide an overview and status of the project and

discuss the approach and mechanism that has been developed to resolve scientific and technical issues, including those surrounding the criteria for toxicity.

Assessment of Risk to Surface Waters Due to Releases from Canadian Copper Smelters and Refineries and Zinc Plants. D.W. Gutzman and P.J. Doyle. Environment Canada, Commercial Chemicals Evaluation Branch, Hull, QC.

The *Canadian Environmental Protection Act* CEPA) requires the federal Minister of the Environment to conduct environmental risk assessments of chemicals, groups of chemicals, effluents and wastes, that have been identified as Priority Substances. Assessments of releases from Canadian Cu smelters and refineries and Zn plants have recently been conducted. Metal-containing particulate matter is released from these facilities into the atmosphere and eventually deposits in terrestrial and aquatic receiving environments. Assessment of the resultant risk to surface waters will be the focus of this presentation. A number of novel approaches were used in these assessments. Based on the Free Ion Activity Model (FIAM), free metal ion concentrations were used as indicators of metal bioavailability. Measured or estimated free ion effect concentrations were, in a very limited number of cases, provided in ecotoxicology publications. The majority of the time they were estimated from information included in the studies, limiting consideration to those studies providing sufficient ancillary data. Stochastic environmental fate and effect modelling was used to estimate the annual flux of soluble metal deposition that would result in steady state free ion concentrations equivalent to the free ion effect concentrations. These annual fluxes are referred to as "critical loads." Stochastic models were based on the range of conditions typical of the Canadian Shield. Simple geochemical speciation models were used to estimate free metal ion concentrations from dissolved concentrations. This approach allowed evaluation of risk based on direct comparison of monitored deposition rates with estimated critical loads.

Hazard Identification of Alloys by Transformation and Toxicity Testing. J.C. Nadeau, J.C. McGeer, J.M. Skeaff, P. King, M. King and S. Brigham. Natural Resources Canada, Metals and the Environment Program, CANMET, Ottawa, ON.

Currently, there is an OECD initiative to develop a globally harmonized system of hazard classification and labeling (HCL). Within this framework, a draft standard laboratory protocol for the hazard identification of metals and sparingly soluble metal compounds in the aquatic environment is being developed. The generalized approach is to measure transformation and dissolution characteristics of metals and metal compounds in standard aqueous media. The draft protocol conditions have yet to be finalized and toxicological validation is required. Transformation tests were performed on powdered forms of cartridge brass, stainless steel and aluminum alloys, as well as comparative mixtures of the pure metals Cu, Zn, Ni, at loadings up to 100 mg/L. The bioavailability and toxicity of the resulting transformation solutions were tested with *Daphnia magna* and *Selenastrum capricornutum* and compared to the results of toxicity tests with soluble metal salts. Results indicate that the stainless steel alloy releases minimal concentrations of Ni and Cr to solution, while brass released concentrations of Cu and Zn significantly lower than would be expected on the basis of a simple Cu-Zn mixture. However once the metal is released into solution, the toxicity is similar to that of the readily soluble metal salts. The conditions under which the transformation/dissolution tests are done can have a dramatic influence on the resulting toxicity.

Protection of Canadian Fish and Wildlife from Mercury. B.M. Miskimmin[1], S.L. Roe[2] and P.-Y. Caux[2]. [1]Limnos Freshwater Consultants, Vernon, BC; [2]Environment Canada, National Guidelines and Standards Office, Hull, QC.

An issue that challenges many environmental resource managers is ensuring the protection of fish and wildlife that consume fish from the toxic effects of Hg. *Canadian Environmental Quality Guidelines* (EQGs) for total Hg and methylmercury provide benchmark levels for water, sediment, and tissue below which adverse effects in aquatic life and wildlife predators of aquatic life are not expected to occur. To develop the EQGs for mercury, a comprehensive assessment of chemical and physical properties, fate, behaviour, Canadian environmental levels, sources, toxicity, and guidelines for other jurisdictions was completed. This information was used according to protocols established by the Canadian Council of Ministers of the Environment (CCME) to derive *Water Quality Guidelines* (WQGs) and *Sediment Quality Guidelines* (SQGs) for the protection of aquatic life and *Tissue Residue Guidelines* (TRGs) for the protection of wildlife consumers of aquatic life. As part of guideline development, the bioaccumulative nature of methylmercury was addressed to ensure that concentrations in water would not result in levels in fish above the TRG. SQGs of 0.17 and 0.13 mg/kg dry weight are recommended for total Hg in freshwater and marine sediments, respectively. A TRG for methylmercury of 33 µg/kg wet weight is recommended for mammalian and avian wildlife. A draft WQG for methylmercury of 0.04 ng/L is proposed for freshwater.

The Mercury Issue in Lake Victoria (East Africa): What is Happening? L.M. Campbell[1], R.E. Hecky[1] and R. Ogutu-Ohwayo[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Fisheries Resources Research Institute, Jinja, Uganda.

Recently, there has been concern about contaminants entering Lake Victoria and its food webs. Considering that the continuation of the successful fisheries is needed to contribute to the emerging economies of the three countries around Lake Victoria, and that most local people on the shores of Lake Victoria depend on fish as a cheap source of protein, it is important to investigate the concentration, extent and source of contaminants in the lake. Here, we briefly examine Hg concentrations in the food webs in Napoleon Gulf (Uganda) and Winam Gulf (Kenya). Hg concentrations vary between trophic levels, for example, Hg concentrations are higher in predacious Nile perch than in benthic feeder Nile tilapia. Hg in Nile perch was similar between the two gulfs. A literature review revealed that Nile perch greater than 5000 g typically contain Hg concentrations exceeding WHO recommended guidelines of 200 µg/kg for at-risk groups, including young children and unborn fetuses, but Nile tilapia Hg concentrations were consistently below the WHO guidelines. Bioaccumulation of Hg in the food webs will be demonstrated and the implications will be discussed.

The Distribution of Total Mercury and Methylmercury in a Lake Ecosystem Using a Mass Balance Approach to Determine the Effect of Intensive Fishing. A. Tremblay[1], C. Surette[2], I. Cartier[2], S. Garceau[2] and L. Cloutier[3]. [1]Hydro-Québec, Hydraulique et Environnement, Montréal, QC; [2]Université du Québec à Montréal, GEOTOP, Montréal, QC; [3] Université de Montréal, Département des sciences biologiques, Montréal, QC.

In reservoirs, concentrations of Hg in all species increased rapidly after impoundment and hence increased the health risks associated with the rise of fish Hg levels. A Few studies done in Finland and in Sweden have demonstrated that intense fishing could significantly reduce the level of Hg in fish. However, the mechanisms responsible for the reduction of the Hg levels are still unknown. Different mechanisms could, in part, explain the reduction of fish mercury concentrations: [1] faster fish growth rate would result in a biodilution effect, [2] a shift in fish diet or a modification of the food web and therefore a change in the source of Hg; and [3] a significant removal of the Hg in the system as we remove the fish. Our study aims to increase our understanding of the mechanisms responsible for the reduction of fish Hg concentrations and the possibility to apply intense fishing on hydroelectric reservoirs and consequently to reduce the health risks associated with the rise of fish Hg levels in reservoirs. Our results showed that lake sediments (first cm only) represent 89-93% of the total Hg

budget and the dissolved fraction (<0.45 µm) represent and another 5-8%. Benthic and planktonic organisms account for less than 1% while the fish contribute from 0.3-1%. As for methylmercury, a metal which bioaccumulates in the food chain, fish represent 12-59% of the methylmercury budget found in lake ecosystems. Sediments, dissolved fraction, benthic and planktonic organisms account for respectively 20-45%, 12-35% and 1-12%. The removal of 25-50% of the fish biomass in a lake represents the removal of around 40% of the methylmercury budget, which is probably enough to explain the reduction in fish Hg level after intense fishing.

PCB Exposure in American Kestrels (*Falco sparverius*): Reproductive, Immunological and Physiological Effects Over Two Generations. J.E. Smits[1], G.R. Bortolotti[2], K.J. Fernie[2] and T. Marchant[2]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Department of Biology, University of Saskatchewan, Saskatoon, SK.

Large die-offs among wildlife due to environmental contamination are becoming relatively rare. Nevertheless, there is a recognized biological cost to animals from contaminant exposure. This occurs primarily at a subclinical level. We define some of those costs in American kestrels (*Falco sparverius*) including [1] directly exposed to dietary PCBs; [2] exposed only *in ovo*; and [3] one year after PCB exposure.

Captive kestrels received 7 mg/kg/d PCBs (a mixture of Aroclor 1248:1254:1260, 1:1:1) through their diet during the breeding season of 1998, which resulted in total PCB levels of 34.0 mg/kg whole egg wet weight. Exposure occurred from 1 month before pairing, until the first egg hatched at approximately 100 d (breeders), or for 120 d (nonbreeding, adult birds).

The PCB exposed breeding birds showed a significant delay in egg laying relative to controls (20.8 vs 14.5 d from pairing, $p=0.003$), and laid smaller clutches. Total clutch infertility was higher in PCB pairs (7 of 25 vs 2 of 25, $p=0.04$), and they laid significantly more small clutches (<5 eggs). Egg composition was also altered, with PCB eggs having relatively larger yolks and less albumen than controls ($p<0.05$). Early post-hatch mortality was dramatically higher in nestlings of contaminated parents (51.7% vs 13.4%), and the number of offspring successfully fledging was also significantly lower ($p=0.05$).

Many of these negative effects were no longer apparent the following year (1999) when the same birds were bred again. However, the *in ovo* exposed offspring from PCB exposed parents, which had been paired with clean, experienced adults of the opposite sex, had a significant delay in egg production. There appeared to be sex specific problems during the breeding season. The pairs with F1 PCB females produced smaller clutches, and 25% of these pairs laid no eggs at all, compared with only 9% of control pairs. After hatching, the pairs with F1 PCB males had higher wastage during the nestling period. Both the peri-hatchling mortality and the percentage of nestlings which fledged successfully were significantly and negatively affected in pairs with *in ovo* exposed males.

In 1998, the thyroid hormone, triiodothyronine (T_3), corticosterone, and plasma carotenoid concentrations showed significant effects of exposure, whereas thyroxine (T_4), estradiol and testosterone did not. Serum T_3 in PCB males was decreased throughout the breeding season ($p=0.04$). In 1999, both the F_0 and the *in ovo* exposed F_1 males continued to show lower serum T_3 levels relative to controls of the same cohort, while T_4 levels were not affected. The corticosteroid levels in PCB males were relatively lower than controls in 1998, and one year later, although baseline corticosteroid levels appeared no different from controls, there was a marked deficit, relative to control cohorts, in their ability to release corticosterone during a multiple sampling stress test.

One aspect of immune function was evaluated in these birds through their humoral (antibody

mediated) immune response. Serum antibody levels were measure against dinitrophenol-keyhole limpet hemocyanin (DNP-KLH), a nonpathogenic antigen with which the kestrels had been vaccinated. In 1998, adult females had significantly higher antibody levels when exposed to PCBs, while the males' levels were significantly suppressed. In 1999, offspring from PCB exposed parents had significantly higher anti-DNP-KLH titres than those from control and ovo-exposed parents. Both B lymphocyte (antibody production) and T lymphocyte responses were altered by PCB exposure. However, enhancement or suppression of immune responses in PCB exposed birds were strongly dependent on age (adult vs fledgling) and sex.

Exposure to dietary PCBs produced changes in reproduction, immune function and endocrine regulation in males and females, with males being generally more severely affected. Several of the reproductive effects were much more pronounced in the first year, leading us to believe that non-persistent PCB congeners have a greater influence on reproduction than do persistent ones. *In ovo* exposure to PCBs resulted in reduced reproductive success, as well as immunomodulation of the humoral immune response. Based upon these observations, it is evident that the endocrine modulating effects of PCBs are operating at a subclinical level, with the potential for negative impacts at the population level.

Mining Environmental Effects Monitoring

Session Co-chairs: K. Hedley and S. McAlpine

Metal Mining Environmental Effects Monitoring. S. Ribey and C. Dumaresq. Environment Canada, National EEM Office, Hull, QC.

The amended *Metal Mining Effluent Regulations* (MMER) will include a requirement for mines to conduct EEM under the authority of the *Fisheries Act*. In December 1999, the Metal Mining EEM Working Group came to final consensus on the mining EEM program. This completed a two-year multi-stakeholder review, which resulted in the development of requirements and technical guidance for the metal mining program. The main elements of the EEM program are a fish population survey and benthic invertebrate community survey, along with a fish tissue analysis. The results of this monitoring will be used to determine if mine effluent is having an effect on the aquatic receiving environment, in particular fish, fish habitat and the usability of fisheries resources. To aid in data interpretation, and possibly determine the cause of effects, mines will also be required to conduct sublethal toxicity testing of effluent twice per year and ongoing monitoring of water and effluent quality four times per year. The metal mining EEM program has been developed to allow site specificity and flexibility, while maintaining scientific integrity within the context of a regulatory program. The program uses a tiered approach to monitoring, where the results of previous monitoring determines what level of monitoring is subsequently required. This includes conducting monitoring at shorter time frames if an effect is found or longer time frames if no effects are found. This talk will include an update on the publication of the MMER in *Canada Gazette*, as well as highlighting the results of the legal review of the EEM requirements, which has occurred.

Streamlining EEMs and Other Monitoring in the Context of a Mine's Life-Cycle. P. Orr, C. Russel and P. Stecko. Phoenix.mg Inc., Toronto, ON.

Environmental monitoring is typically required throughout the various stages of a mine's life-cycle, however the elements of a monitoring program often fail to evolve in response to the different information requirements associated with each life-stage. The scope of monitoring studies frequently expands over time without a commensurate decrease in requirements that are no longer relevant.

New regulations respecting Environmental Effects Monitoring (EEM) will involve new activities and expense for many mines and will need to be addressed in the larger context of a mine's life-cycle and corporate environmental commitments. Ideally, a monitoring program should address corporate environmental objectives, satisfy multiple stakeholders and regulations, and yet be as streamlined as possible to optimize cost-effectiveness. This presentation will highlight the critical objectives and considerations associated with environmental monitoring during mine development through to decommissioning and outline triggers for altering the elements of environmental programs to meet the evolving needs. Case examples will be presented highlighting the improved environmental understanding and concurrent cost savings that have been realized by mines that have revamped their monitoring programs accordingly. The role of future EEM studies will be considered in the context of the overall monitoring strategy of mines at different stages of development.

Developing a Wabush Lake Tailings Management Plan: A Multi-Step, Multi-Stakeholder Approach. L. Preziosi[1], E. Luiker[2] and D. Farara[3]. [1]Iron Ore Company of Canada, Labrador City, NF; [2]Jacques Whitford Environment Ltd., St. John's, NF; [3]BEAK Consultants, Brampton, ON.

The Iron Ore Company of Canada (IOC) is the largest iron ore producer in Canada and operates large open pit mines and processing plants at its Carol Project in Western Labrador. Wabush Lake forms the eastern boundary of the Carol Project and has received approximately 630 million tonnes of tailings from plant operations over the past 38 years. The tailings are not acid generating. The residual iron minerals in the tailings significantly discolour the receiving lake, at peak flows this "red water" plume can extend some 30 km downstream of IOC's discharge point. This has led to a significant impact on the ecological and recreational values of Wabush Lake and downstream environments which has caused considerable community concern. Federal regulations (MMLER) were introduced by Environment Canada in 1977 to regulate effluent discharges from mining operations. These regulations only applied to new mines that started operation after 1977. IOC was therefore exempt from MMLER although other federal requirements under the *Fisheries Act* may apply. Presently IOC is only 1 of 2 mines in Canada with Provincial approval for unconfined tailings disposal. The Federal Government is proposing new *Metal Mining Effluent Regulations*, which will apply to all mines, including IOC operations. The new regulations will require IOC to confine tailings release. Presently, IOC's tailings discharge meets proposed MMER limits, with the exception of total suspended solids (TSS). This presentation is divided into three parts. The first outlines the regulatory and community issues and need for change. As well, it will outline the process established to engage key stakeholders and to evaluate options. The second part will detail the existing environmental conditions in Wabush Lake. The third part ties the two together to develop the Tailings Management Plan which will comply with regulatory and community requirements and to mitigate or minimise current impacts to the receiving environment.

Environmental Effects Monitoring at a Gold Mine in Northern Ontario. C.D. Wren[1], N. Hartrupp[1], M. Bednarz[2] and K. Ferguson[3]. [1]ESG International, Guelph, ON; [2]Placer Dome North America, South Porcupine, ON; [3]Placer Dome North America, Vancouver, BC.

Detailed aquatic assessments were conducted at the Detour Lake mine site in 1995 and 1998. The Detour Lake gold mine opened in 1983 and ceased operation in late 1999. The aquatic surveys included 22 stations from three exposure lakes and two reference lakes, as well as creeks and rivers. Traditionally, Cu and cyanide have been the chemicals of most concern at the site. Effluent from the ore processing was originally treated with hydrogen peroxide, however, improved effluent quality was obtained by installation of a SO_2/air slurry treatment plant in 1995. The treated effluent is now non lethal to rainbow trout and *Daphnia magna*. Effluent quality improved considerably between 1995 and 1998. Consequently, water quality in the receiving environments also improved. The concentration

of all metals, except Cu, are below the *Provincial Water Quality Objectives* in the receiving environment. Sediment metal concentrations were higher downstream of the mine compared with reference sites. The sediment quality Severe Effect Level (SEL) was exceeded for Ni and Cu at some sites. The benthic community was affected below the discharge and showed recovery with distance downstream of the mine site. Phytoplankton biomass and diversity was lower in the downstream exposure lakes, but zooplankton community structure was not affected. The fish community was less diverse in lakes exposed to mine effluent in 1995 but not 1998. The data show an excellent gradient of responses of the aquatic ecosystem relative to water and sediment chemistry. In addition, there were demonstrated improvements in biological variables in response to improved effluent quality.

EEM Study Associated with 11 Closed Mine Sites at Elliot Lake, Ontario. P. Stecko[1], C. Russel[1], R. Payne[2], A. Coggan[2] and I. Ludgate[3]. [1]Phoenix.mg Inc., Toronto, ON; [2]Rio Algom Ltd., Elliot Lake, ON; [3]Denison Mines Ltd., Elliot Lake, ON.

Anticipated changes to the *Fisheries Act* will require operating mines to undertake Environmental Effects Monitoring (EEM) studies to assess the degree and extent of mine-related impacts on fish, fish habitat and the use of fisheries resources. Although closed mines will not be included among the mines required to undertake EEMs, Rio Algom Limited and Denison Mines Limited elected to undertake a comprehensive receiving environment study in 1999 in the vicinity of their closed mine sites near Elliot Lake, Ontario. Through developing and implementing a watershed program, the mines were able to address EEM monitoring for 11 closed mines in one study. The approach allows for a more effective allocation of resources and an integrated assessment of spatial effects/improvements associated with mine decommissioning. The study encompassed a large portion of the Serpent River watershed, a system of chain-lakes and streams draining an area of approximately 1,376 km^2. Samples of water, sediment, benthic invertebrates and fish were collected from 20 stations distributed throughout the watershed representing near-field, far field and reference locations. Preliminary results of the study will be presented together with a review of the study design and the successes and pitfalls of an EEM approach on an inland lake system.

Impact of Mine Tailings in the Marine Environment: Preliminary Observations at a Site in Little Bay, Newfoundland. G. Veinott[1], J. Meade[1], C. Andrews[1], L. Fancey[1], F. Power[1], C. Stirling[1], P.J. Sylvester[2], M.R. Anderson[1] and J.F. Payne[1]. [1]Department of Fisheries and Oceans, St. John's, NF; [2]Memorial University of Newfoundland, St. John's, NF.

There are major policy decisions surrounding the comparative risks of disposal of mine tailings in freshwater or marine environments. Preliminary chemical, physical and ecotoxicological studies have been carried out on tailings which entered Little Bay some years ago from an abandoned copper mine. Impact zone delineation and determination of volume of tailings in the Bay is being carried out with novel hydroacoustic techniques as well as sediment chemistry. Concentrations of various metals in sediments in the Bay are elevated and reflect a tailings source but anomalous results have been obtained for levels of lead in certain areas. With respect to body burdens in biota, concentrations of several metals are distinctly elevated in mussels (*Mytilus sp.*), clams (*Mya arenaria*) and seaweed (*Fucus vesiculosus* and *Ascophyllum nodosum*) in Little Bay in comparison with species from a control site. Concentrations of metals were generally higher in mussels than in clams. A greater range of metals was also found in *Fucus* than in *Ascophyllum*. However both bivalves and seaweeds are abundant at the site indicating that any population level effects on these particular organisms are not overt. Extensive trawling in the area indicated the presence of few fish or macrobenthos but it is premature at this time to suggest possible causes such as habitat avoidance or habitat toxicity. However, both physical and chemical toxicity has been established for "weathered" tailings in the Bay through use of the amphipod bioassay indicating the potential for impact on sediment communities.

(Study supported by the Toxic Substances Research Initiative, TSRI).

The Toxicity of an Effluent Produced by a Diamond Mine in the Northwest Territories to Freshwater Zooplankton. S.J. Crocquet de Rosemond[1], K. Liber [1], D. Waite[2] and S. Harbicht[3]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Environment Canada, Environmental Conservation Branch, Regina, SK; [3]Environment Canada, Environmental Protection Branch, Yellowknife, NT.

The Ekati Diamond Mine, NT, manually extracts diamonds from kimberlite using chemical free processes that reduces the ore to fines (0.5 mm). An effluent consisting of the fines, coagulants, flocculents and the waste water employed during the extraction process is pumped to the Long Lake Containment Area. Examination of field data indicates that native zooplankton populations have been adversely impacted by the addition of the effluent to the Containment Area. In laboratory experiments, the effluent did not prove to be acutely toxic to *Ceriodaphnia dubia*, however, a series of chronic toxicity tests (7 d) revealed that the filtered effluent significantly affected survival at concentrations of approximately 50%, and reproduction at concentrations as low as 12.5-25%. Currently, a series of toxicity identification evaluations (TIE) are underway to investigate the toxic nature of the effluent to *C. dubia*. A TIE using EDTA as a chelator failed to reduce toxicity of 100% and 50% effluent to *C. dubia*, indicating toxicity may not be caused by certain cationic metals. The toxicity of 100% and 50% effluent was partially reduced by adjusting the pH to 6.5, which indicates that toxicity may be, at least partially, caused by pH-dependent toxicants such as ammonia. Additional Phase I TIE experiments are currently underway to identify components of the effluent that may be responsible for toxicity.

Optimizing Sample Location Selection in Environmental Effects Monitoring (EEM) Programs. M.D. Paine. Paine, Ledge and Associates (PLA), North Vancouver, BC.

In environmental effects monitoring (EEM) programs, response variables (*Y*) of interest are compared among locations representing different degrees or levels of exposure to a point or non-point source. The levels of exposure can be qualitative (e.g., reference, near-field, far-field) or quantitative (e.g., % effluent; concentrations of one or more contaminants; distance and direction from the source). ANOVA or regression models can be used for study design and data analyses. In either case, the exposure levels are effectively *X* variables, and statistical power will differ among different distributions or sets of exposure levels and locations. Extreme distributions, with most or all locations representing either minimum or maximum exposure, will maximize power for overall *F* tests in ANOVA or regression. However, extreme distributions may not be the most powerful for other hypothesis tests or comparisons of interest. Extreme distributions are also rarely useful for estimating effects at intermediate exposure levels, and the spatial extent of effects. Uniform or evenly spaced distributions are usually the most flexible, robust and informative. Clumped uniform distributions, with each of a few exposure levels represented by >1 location, can combine the flexibility and robustness of uniform distributions with the power of extreme distributions. Centred distributions (e.g., normal distributions), with most exposure levels intermediate, are the least powerful, and should generally be avoided.

Metal Mining Environmental Effects Monitoring - Small-Bodied Adult Fish Survey. A. Rosaasen, K. Himbeault and J.D. Embury. Conor Pacific Environmental Technologies Inc. Saskatoon, SK.

Alternatives to large-bodied adult fish surveys are available in the Metal Mining Environmental Effects Monitoring program. In small receiving water lakes, population survey sample requirements for large fish species can have a detrimental effect on the sampled population, and potentially confound the study design. To minimize such concerns, small-bodied fish species were evaluated as alternative

sentinel species in a small waterbody located immediately downstream of a uranium mine and mill effluent reservoir, and at a reference location. The results of the survey, the constraints of the study design and the selection criteria for the small-bodied sentinel species will be discussed.

Simplified Prey Communities Lead to Energetic Bottlenecks for Yellow Perch Inhabiting Metal-Contaminated Lakes. G.D. Sherwood[1], J. Kovecses[1], A. Hontela[2] and J.B. Rasmussen[1]. [1]Department of Biology, McGill University, Montreal, QC; [2]Université du Québec à Montréal, Montréal, QC.

Heavy metal loading into aquatic systems, with its potential for adverse effects on receiving biota, has been the subject of numerous studies from a wide range of disciplines including fish physiology and community ecology. However, despite the broad interest base, very few studies have been successful in providing plausible, mechanistic links between different levels of biological response to metals (e.g., physiological to community or vice versa). Here, we present a trophic-based approach to describing energy relationships in metal-contaminated lakes, emphasizing the importance of food-web complexity in maintaining a positive energy balance in resident fish. Both diet and community analysis indicated that the food base leading to yellow perch (*Perca flavescens*) in metal-impacted lakes was extremely simplified compared to that found in reference lakes; affected communities consisted of only a limited choice of small, metal-tolerant prey taxa such as zooplankton and chironomids. Through the application of an *in-situ* marker for burst swimming/foraging costs (muscle lactate dehydrogenase activity) and through bioenergetic modelling, we show how this has severe consequences on the efficiency of energy transfer to perch from their prey; premature energetic bottlenecks (zero growth resulting from high swimming costs) occur when successively larger prey types are not available to growing perch. These observations provide a plausible framework within which physiological (perch growth) and ecological (prey community) responses to heavy metal loading can be linked.

Seasonal Variation of Intermediary Metabolism and Hormonal Status of Adult Yellow Perch (*Perca flavescens*) Chronically Exposed to Metals. H. Levesque[1], A. Hontela[1], T.W. Moon[2], G.J. Van Der Kraak[3] and P.G.C. Campbell[4]. [1]Département des Sciences Biologiques, Université du Québec à Montréal, Toxen Research Centre, Montréal, QC; [2]Department of Biology, University of Ottawa, Ottawa, ON; [3]Department of Zoology, University of Guelph, Guelph, ON; [4]INRS-Eau, Université du Québec, Ste. Foy, QC.

The effects of heavy metals on lipid and carbohydrate metabolism and hormonal status were investigated in yellow perch from a mining area in northern Québec. Fish from six lakes situated along a contamination gradient of Cd, Zn and Cu were captured and subjected to a confinement stress of 1 h, in fall 1998 and summer 1999. Fish from contaminated lakes had an impaired capacity to increase blood cortisol and glucose following the stress test and exhibited a delayed gonadal recrudescence compared to fish from reference lakes. In the summer, fish from the most contaminated lakes had higher plasma free fatty acid levels, lower reserves of hepatic glycogen and triglycerides, and lower activity of ME and G6PDH, two key enzymes of intermediary metabolism. In the fall, fish from contaminated lakes had higher glycogen reserves and higher activity of PyK, a glycolytic enzyme, and GOT and MDH, two gluconeogenic enzymes, than fish from reference lakes. These results indicate that chronic exposure to sublethal levels of heavy metals has a significant impact on the metabolic and physiological status of yellow perch. The mechanisms through which the metabolic alterations are mediated are under investigation. (Funded by CNTC and MITE-RN).

The Importance of Dissolved Organic Matter to Metal Interactions at Fish Gills. R.C. Playle[1], M.L. Schwartz[1], P.J. Curtis[2] and B.K. Burnison[3]. [1]Department of Biology, Wilfrid Laurier University,

Waterloo, ON; [2]Faculty of Science, Okanagan University College, Kelowna, BC; [3]Environment Canada, National Water Research Institute, Burlington, ON.

Negatively charged dissolved organic matter (DOM) reduces acute metal toxicity to fish by binding metal cations. Modelling metal interactions at freshwater fish gills reflects the importance of DOM concentration on the protection seen against different metals. For example, a Cu-DOM conditional equilibrium binding constant of log K =9.1 means that organic matter keeps Cu off the gills well, because the Cu-gill binding constant is fifty times lower (log K =7.4); a similar situation exists for Pb. Conversely, organic matter keeps Cd off fish gills poorly, because its binding constant is log K =7.4, sixteen times lower than that of Cd to the gills (log K =8.6); a similar situation exists for Ag. On a finer scale DOM quality affects the protection by DOM against metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and against metal accumulation by their gills. We are working on better defining these DOM quality factors as they are related to DOM source (e.g., allochthonous versus autochthonous DOM) and to DOM fate (e.g., as affected through degradation by light and by bacteria).

Relative Protective Effects of Different Dissolved Organic Matters Against Cu, Pb, and Cd Accumulation by Gills of Rainbow Trout (*Oncorhynchus mykiss*). M.L. Schwartz[1], R.C. Playle[2] and P.J. Curtis[3]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [3]Faculty of Science, Okanagan University College, Kelowna, BC.

One of the most important ligands available in natural surface waters that can bind metals is dissolved organic matter (DOM). Not all DOMs are the same, however, and their differences can alter their effectiveness in tying up metals and rendering metals less toxic to aquatic organisms. We isolated DOM by reverse osmosis from a variety of locations, to include mostly allochthonous DOM (e.g., organic material washed into a water body) to mostly autochthonous DOM (e.g., organic material produced within the water column). Trout (~1 g) were exposed to ~0.4 µM Cu, Pb, and Cd in a mixed metal solution plus DOM, toxicity was determined, and metal accumulation by their gills was assayed by graphite furnace atomic absorption spectrophotometry. Generally, darker coloured allochthonous DOM protected better against metal toxicity and accumulation than did lighter, more autochthonous-like DOM. Ca in the water also offered good additive protection, particularly against Pb and Cd accumulation by trout gills.

Creating a Model for Predicting Metal Toxicity to Invertebrates. J. Schroeder[1,2], U. Borgmann[3] and D.G. Dixon[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Ontario Ministry of the Environment, Etobiocke, ON; [3]Environment Canada, National Water Research Institute, Burlington, ON.

Standards for the protection of the aquatic environment are generally limited to total metal concentrations even though they do not reflect metal bioavailability. To address differences in metal bioavailability, research is underway to create computer models that can predict toxicity of metals under various physical/chemical conditions. Recently, the Biotic Ligand Model was developed to predict acute toxicity of a few metals to fish by including the fish gill in the model as a biotic ligand. As part of the national MITE-RN (Metals in the Environment Research Network) program, our study attempts to predict toxicity of metals to *Hyalella azteca* by investigating the relationship between observed toxicity and measured concentrations of metal in water and tissue. Preliminary work has included acute toxicity tests using *Hyalella* exposed to single metals in media of differing physical/chemical characteristics using organisms pre-acclimated to both standard and adjusted medium. The next stage of the study will be to expose suitably-acclimated organisms to selected concentrations of metal in defined media. Individual ions in the artificial media will be varied and linked

to metal accumulation by the organism. A summary of the study and results of tests completed to date will be presented.

Environmental Control Programs at Richmont Mines Inc. Newfoundland Operations - Nugget Pond Gold Mine and Hammerdown Gold Deposit. S. McAlpine, Richmont Mines Inc., Baie Verte, NF.

Nugget Pond is the only operating gold mine in Newfoundland (1997 to present). In late March of 2000, Richmont Mines purchased the Hammerdown Gold Deposit. Ore from Hammerdown will be transported 142 km for processing to Richmont's Nugget Pond Mine/Mill. The joint operation will continue for a minimum of seven more years. Safety, Environmental and Production results since the start of commercial production at Nugget Pond have met expectations and been economical in spite of falling gold prices. The Nugget Pond gold mining project has created more than 90 direct jobs in rural Newfoundland, more than 95% being native Newfoundlanders. This number will increase with the addition of Hammerdown.

Recognition: In 1998, the Minister of Environment and Labour presented a letter of commendation for an excellent environmental management record at Nugget Pond." In 1998 and 2000, Richmont Mines and the mine manager each received provincial environmental awards recognising outstanding service in the protection and preservation of the Newfoundland and Labrador environment. In March of 2000, Richmont received a national environmental award from the Prospectors and Developers Association of Canada. During May 2000, the Nugget Pond Gold Mine received the John T. Ryan Special Award (national safety), in recognition of notable achievement in safe mining.

Environment (Nugget Pond): Richmont Mines have given considerable thought to environmental issues. Compliance and baseline water quality monitoring programs have been in place since 1994. Key aspects of tailings' disposal and wastewater control at Nugget Pond Mine include: [1] all waste rock is being recycled underground as backfill; [2] cyanide destruction is by the INCO SO_2 air destruction process; [3] tailings are submerged in a tailings pond (acid-drainage will not be an issue); [4] daily environmental sampling is carried out with analysis conducted both by Richmont's on-site lab and by a fully accredited off-site commercial lab; and [5] since the start of the mill operation, all samples indicate complete compliance with the Environmental Certificate of Approval for the operation. Richmont Mines has developed a reclamation plan for the Nugget Pond property reflecting provincial guidelines contained in the Certificate of Approval. Some reclamation work including tree and grass planting, recycling and surface contouring has been completed even at this early stage of the operation.

Environment (Hammerdown): Richmont intends to develop and operate the Hammerdown Mine with the same level of care and attention to environmental control as demonstrated at Nugget Pond. The test mining program planned for 2000 includes reclamation of several hectares of area disturbed during previous exploration and logging campaigns. Baseline environmental sample results, wildlife surveys have been conducted and test results show that the ore has a neutral to basic pH. Hammerdown is less than 100 hectares in size and only a small portion of that will be disturbed. There are no continuous flowing streams in the area. A large settling sump was built to help settle any suspended solids. Richmont proposed that the province apply the proposed federal criteria for mine water effluent discharges to the Certificate of approval. This makes the Hammerdown project the first in Canada permitted with the *Metal Mine Effluent Regulations*. The demonstrated performance of the Nugget Pond Mine and Mill indicates a strong beginning and a sound future for the operation and is a tribute to all who contributed to the planning, financing, construction, start-up, operations and management.

Sediment and Soil Toxicity Testing

Session Co-chairs: R.P. Scroggins and G.L. Stephenson

Ecotoxicological Test Methods in Soil: Current Status and New Examples. J. Römbke. ECT Oekotoxikologie GmbH, Boettgerstr. 2-14, D-65439 Flörsheim, Germany.

ABSTRACT

As part of the European notification or registration process for chemicals, their ecotoxicological hazard potential in soil has to be evaluated. Until recently, only a small number of standardised (mainly acute) tests with earthworms and plants was available. Especially methods with chronic endpoints are missing. Moreover, beyond structural aspects, the question of possible influences of chemicals on ecological soil functions should be the focus of interest.

This contribution provides an overview of existing methodological approaches, primarily on zoological and functional test methods. The Enchytraeid Reproduction Test and the Litter Degradation Test potentially required for the registration of pesticides will be presented as examples for a laboratory and a field test, respectively.

All test methods mentioned so far are primarily related to the evaluation of individual chemicals. These (modified) biological test methods can be used in the area of "Soil Quality Assessment", where the contamination of a field soil is often unknown. However, no method covering especially the evaluation of potentially contaminated field soils has been standardised in Europe so far.

INTRODUCTION

As part of the European notification or registration process for chemicals, their ecotoxicological hazard potential in soil has to be evaluated. Until recently, only a small number of standardised (mainly acute) tests with earthworms and plants was available. However, more and more often terrestrial effect tests are required if exposure of soil organisms is probable. For example, the following European laws or guidelines contain such requirements : [1] Pesticides: EU Guideline 91/414 (1991) and several recent adaptations e.g., tests with micro-organimss, plants, earthworms, arthropods, or on decomposition; [2] Industrial chemicals: EU Technical guidance document (1996); under review, e.g., tests with plants and earthworms; potentially more (e.g., microcosms); [3] Biocides: EU Guideline 98/8 (1998); work started just recently, e.g., tests with plants and earthworms; potentially more; and [4] Veterinary drugs: EU EMEA/CVMP/055/96 (1997) potentially many tests possible, but in reality rarely required.

All requirements listed so far refer to the assessment of individual chemicals. However, according to German federal soil protection law (BBodSchG 1998), it is possible to assess the quality (i.e. its potential to act as a habitat for organisms) of the soil at a certain site by using ecotoxicological tests. Unfortunately, no details are provided by the German government yet which tests should be used when. It is clear that relatively easy the methods related to the evaluation of individual chemicals can be modified. So, the tests are useful for the purpose of "Soil Quality Assessment", where the contamination of a field soil is often unknown. No method focussing especially on the evaluation of potentially contaminated field soils has been standardised in Europe so far.

Overview on existing terrestrial test systems.

Criteria for the selection of the presented test systems:

In the following, ecotoxicological test systems will be listed that are: [1] Standardised (preferably validated) and well documented in the literature; [2] Internationally accepted, published by organisations like BBA, EPA or OECD; and [3] Functional tests, since these tests are performed on

the ecosystem level.

The following ecotoxicological test systems will not be discussed: [1] Biomarkers and other tests on the biochemical or cellular level since extrapolation of the results of these tests to the ecosystem level are difficult (if possible at all); [2] Genotoxicity and mutageneity tests since they are mainly aiming at human toxicology; and [3] Mammalian or avian test methods since they are similar to toxicological methods. In general, botanical, microbiological as well as zoological test methods are covered.

Historical overview of terrestrial test development:
In Fig. 1, the number of tests proposed annually between 1980 and 2000 is shown. It is obvious, that during the Eighties (with the exception of some very important tests published by OECD in 1984) only few tests were developed. Later on, in parallel to growing legal requirements the number of test proposals increased considerably. Especially in the project SECOFASE, sponsored by the European Union until 1996, nearly 20 already well standardised tests methods have been developed (Løkke and van Gestel 1998). Currently, the number of new tests has reached a more or less constant level of about five per year. In addition, it is shown in Fig. 1 that nearly all tests are performed in the laboratory (81%), whereas only 13% and 6%, respectively, of the tests belong to the semi-field or field level.

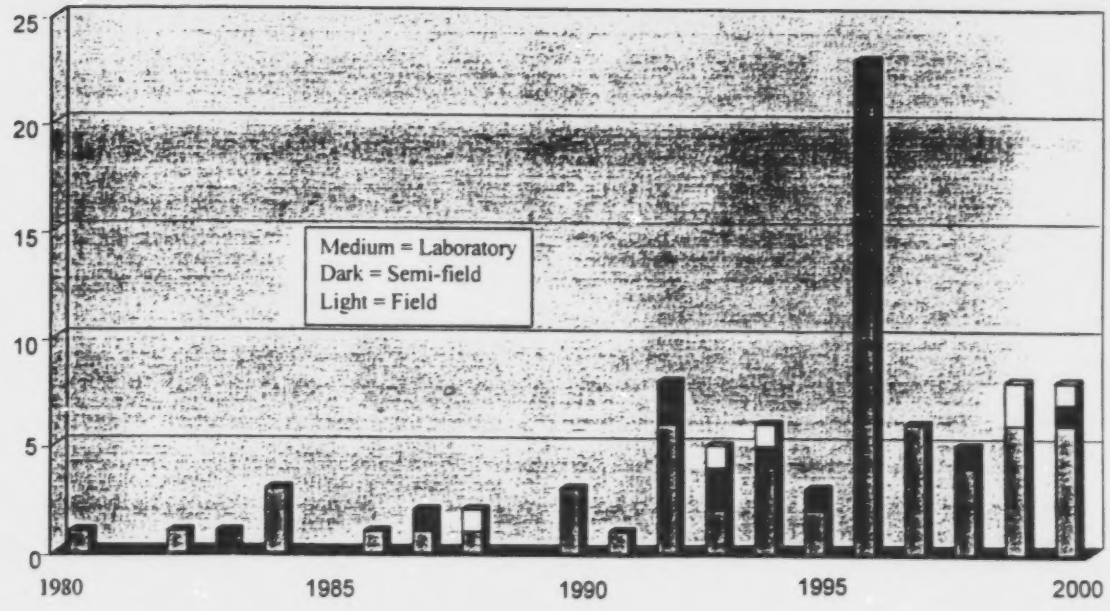

Fig. 1. Number of terrestrial ecotoxicological test systems proposed between 1980 and 2000 including a differentiation between tests in the laboratory, on the semi-field level or directly in the field.

In Table 1, the total number of terrestrial tests is classified according to the taxonomic group to which the test organism belongs. The latter are also roughly allied to a certain trophical group. It is obvious that some groups (especially the saprophagous oligochaete worms) are quite good represented whereas tests with plants and predators are not very abundant. Herbivorous species are missing completely.

Table 1. Number of tests for some important soil organism groups (including the trophical group to which the test organism belongs).

Taxonomic group	Number of tests	Trophic group
Plants	13	Producers
Micro-organisms	12	Mineraliser etc.
Nematoda	8	Diverse
Oligochaeta	15	Saprophagous
Collembola	8	Saprophagous
Coleoptera	7	Predators (mainly)
Arachnida	5	Predators
Other Arthropoda	5	Diverse

Examples of effect test systems in the laboratory:
In the listing, the most important existing terrestrial tests are shown. In addition to the guideline in which the test is described it is mentioned which other organisations have published the same or very similar test method:

Microbial Soil Respiration Test: BBA VI, 1-1 (1994); OECD Draft
Determination of Nitrogen Processes: ISO 14238 (1997)
Acute Plant Emergence Inhibition Test: OECD 208 (1984); EPA, ISO
Chronic Plant Reproduction Test: ISO Draft (2000); ASTM
Earthworm Acute Mortality Test: OECD 207 (1984); FDA, ISO
Earthworm Reproduction Test: ISO 11 268-1 (1998); BBA
Enchytraeid Reproduction Test: OECD 220 Draft (2000); ASTM, ISO
Collembola Reproduction Test: ISO 11 269 (1999)
Acute Carabid Beetle Mortality Test: BBA VI, 23.-2.1.8 (1994); IOBC
Chronic Staphylinid Reproduction Test: BBA VI, 23.-2.1.108 (1994); IOBC

Example of a laboratory test: Enchytraeid Reproduction Test (ERT):
Especially tests methods with chronic endpoints are missing so far. Therefore, as an example of a new chronic test the main properties of the ERT are described (Römbke and Moser 1999):

Status: Draft guideline according to OECD, ISO and ASTM standards
Test Species: *Enchytraeus albidus*; *Enchytraeus sp.*; 10 adult worms
Substrate: Artificial Soil according to OECD (1984a)
Test Design: Range-Finding Test: 2 weeks.; Definitive Test: 6 weeks: Removal of adults after 3 and counting of juveniles after 6 weeks
Parameter: Mortality, behaviour, number of juveniles:
Performance: Temperature 20±2 °C; weekly feeding with rolled oats
Concentration: NOEC: 5 concentrations; 4 (control: 8) replicates; ANOVA ECx: ≈ 12 concentrations; 2 (control: 6) replicates; Probit
Reference Substance: Carbendazim (trade formulation Derosal)
Validity (Control): Mortality <20 %; juveniles >25/vessel; CV (repro.) <50%

Examples of tests on higher investigation levels:
Since it is not the main focus of this contribution, tests on the semi-field or field level are not presented (for details see Scott-Fordsmand, this volume). However, some examples are listed in the following:
Semi-field methods:

Chronic effects on isopods and litter decomposition (Van Wensem 1993);
Disturbed Soil Microcosms (Ohio System) (Parmelee *et al.* 1993);
Terrestrial Model Ecosystems (TME) (UBA 1994);

Mortality / Feeding rate of Carabid Beetles (IOBC Draft Guideline 2000)
Field method:
Abundance and diversity of Earthworms (BBA Guideline VI, 2-3, 1994)

Functional tests – a new area of terrestrial ecotoxicology.
Ideas for ecosystem (i.e. functional) test methods:
Functional methods are used to test the effects of a chemical on high levels of biological organisation, using organic matter degradation or nutrient cycling as measurement endpoint.

On the contrary structural tests are performed with individuals or populations of organisms. For a complete ecotoxicological assessment of a chemical functional test methods are necessary since only these really measure ecological effects (i.e. on the ecosystem level). Functional test methods with a high potential for standardisation are given below (including the main measurement endpoint):
Litterbag tests: Organic matter degradation (Kula and Römbke 1998)
Bait-Lamina test: Biological (feeding) activity (Von Törne 1990)

Example of a field test: Litter Decomposition Test:
The Litter Decomposition Test, which is becoming more and more required for the registration of pesticides in Europe (despite the fact that it has not been standardised formally so far), is presented in the following:
Requirement: EU Pesticides Guideline 91/414 (1991), Annex III, 10.6
Original method: Bocock and Gilbert (1957), Crossley and Hoglund (1962), Dunger and Fiedler (1997), Eisenbeis (1994)
Guidance paper: Kula and Römbke (1998), Kula (personal comm.)
Performance:
Principle: Mass loss of organic material enclosed in (litter) bags
Material: Natural: e.g. straw, hay, tree litter. Artificial: Cellulose
Exposure: On the soil surface or buried in the soil (0–5 cm)
Duration: Usually up to one year (four sampling dates)
Application of chemicals: According to agricultural practice or at plateau concentration
Evaluation: Comparison with control or reference substance.

Currently it is not known whether this test, preferably after an international ringtest, will be validated formally as part of the standardisation process which could be organised by OECD or ISO.

DISCUSSION

In the following it is tried to summarise the current status of terrestrial ecotoxicological test methods. This summary can be divided in three parts:
Current status of acceptance:
Many (mainly European) laws require terrestrial tests (usually few).
Micro-organisms, plants and earthworm tests are widely accepted.
Situation of test method development:
Ca. 50 laboratory, approx. 10 semi-field but nearly no field studies (just 3) are standardised or even validated.
Requirements and assessment for new test methods are agreed on.

Needs for the future in terrestrial ecotoxicological test methodology:
Many test methods are not yet fully standardised and validated.
Functional test methods must be further developed.
Most urgently needed: An international agreement about test strategies: which test has to be used when for which purpose? Despite several proposals such a paper is not within close sight.

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Note: The references for the guidelines mentioned in this article are not included in this list. They can be ordered directly from the issuing organisations.

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Folsomia candida: **A Good Organism for Soil Ecotoxicity Testing?** K. Becker-van Slooten, C. Staempfli and J. Tarradellas. DGR–GECOS–Ecotoxicologie, Ecole polytechnique fédérale de Lausanne, 1015 Lausanne, Switzerland.

There is an increasing need for appropriate methods which allow to determine the soil quality and the effects of xenobiotics. Soil ecotoxicology being a relatively young science, there are only two standard bioassays with soil invertebrates described in international guidelines and used for routine determination: one concerning the earthworm *Eisena fetida* and since recently one with the springtail *Folsomia candida* (ISO/FDIS 11267, Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants). *F. candida* is a parthenogenic species of ecological importance. These springtails can easily be bred in the laboratory and their biology and ecology is quite well known. According to the ISO protocol, the organisms are exposed to a contaminated standard artificial soil and the number of

offsprings is recorded after 28 d. Some difficulties as well as the advantages and disadvantages of *F. candida* and of the ISO protocol will be presented. In order to develop predictive early warning methods and in order to establish a relationship between a long term (reproduction) and a short term effect, we study the effect of pollutants on a subcellular level by using biochemical biomarkers. Induction of a heat shock protein (hsp 70) is followed as a stress marker and protein, lipid and carbohydrate contents are measured as metabolism markers. The results and the interest of such an approach will be discussed.

Toxicity of Copper Sulphate to Plants, Earthworms, and Springtails in Artificial and Field-Collected Soils. N.C. Feisthauer[1], G.L. Stephenson[1], J.I. Princz[2] and R.P. Scroggins[3]. [1]ESG International, Guelph, ON, [2]University of Guelph, Guelph, ON, [3]Environment Canada, Ottawa, ON.

As part of Environment Canada's continued development of soil toxicity testing methods, the performance of a battery of terrestrial toxicity tests was assessed by exposing the test organisms to soil spiked with copper sulphate ($CuSO_4$). The test battery included acute and chronic tests with two earthworm, one springtail and six plant species, in both artificial and three field-collected soils (a clay loam, sandy loam and a silt loam). The assessment endpoints included survival, behavioural responses, biomass, growth and reproduction metrics. $CuSO_4$ toxicity to plants was influenced by species, endpoint, soil type, soil pH, and test duration. Most acute and chronic IC_{50}s for plant species ranged from 1,000-8,000 mg $CuSO_4$/kg soil d.w. $CuSO_4$ toxicity to earthworms was influenced by soil type and test duration. Acute (14 d) LC_{50}s ranged from 1,498-4,147 and 787-1,743 mg $CuSO_4$/kg soil d.w. for *Eisenia fetida* and *Lumbricus terrestris*, respectively. EC_{50}s for *E. fetida* reproductive endpoints were an order of magnitude lower than acute LC_{50}s. Regardless of soil type, both earthworm species avoided soil spiked at sublethal concentrations of copper sulphate. $CuSO_4$ was not acutely toxic to adult springtails (*Onychiurus folsomi*) following 7 d exposures to concentrations up to 10,000 mg $CuSO_4$/kg soil d.w. However, chronic (35 d) exposure to $CuSO_4$ resulted in adult springtail LC_{50}s of 7,305-11,814 (extrapolated) mg CuSO4/kg soil d.w. EC_{50}s for adverse effects on springtail reproduction were 1.7-2.6 times less than adult LC_{50}s.

Development of a New Environment Canada Test Method for Measuring Emergence and Growth in Soil Using Terrestrial Plants. J.A. Miller[1], R.P. Scroggins[2] and G.L. Stephenson[3]. [1]Miller Environmental Sciences Inc., Stroud, ON; [2]Environment Canada, Method Development & Application Section, Ottawa, ON; [3]Aquaterra Environmental, Orton, ON.

In 1994, Environment Canada (EC), the Canadian Association of Petroleum Producers (CAPP) and the Federal Panel for Energy Research and Development (PERD) initiated a multi-year program to develop biological test methods that could be used to assess the toxicity of contaminants in soils using terrestrial organisms. The goal was to develop test procedures that were applicable to Canadian soil types using terrestrial species that were representative of Canadian soil ecosystems. The creation of a new Environment Canada test method, "Test for Emergence and Growth in Soil Using Terrestrial Plants" is the final phase of the soil toxicity test method development program. The new terrestrial plant toxicity test is based on research conducted by ESG International and the University of Guelph and includes procedures for both an acute screening test and a definitive chronic toxicity test with seedling emergence (survivorship) and plant growth/vigour (shoot/root wet mass, dry mass, and length) as endpoints. The method also includes procedures for a short-term (6-8 d) acute reference toxicant test. Six species are recommended in the method including: barley, corn, cucumber, northern wheatgrass, radish and alfalfa (3 dicots and 3 monocots). Key components of the test procedure and rationale for selection of test specifications will be discussed.

Environment Canada's Evolving Biological Test Methods for Soil Toxicity to Earthworms (*Eisenia sp.* or *Lumbricus terrestris*). D.J. McLeay[1], G.L. Stephenson[2] and R.P. Scroggins[3]. [1]McLeay Environmental Ltd., Victoria, BC; [2]Aquaterra Environmental, Orton, ON; [3]Method Development and Application Section, Environment Canada, Ottawa, ON.

In 1994, Environment Canada's Method Development and Application Section initiated a multi-year program to research, develop, validate, and publish a number of standardized biological test methods for measuring the toxicity of samples of contaminated or potentially-contaminated soil, using appropriate species of terrestrial test organisms. This program has included the development of the following three test methods for soil toxicity assessment using earthworms (*Eisenia sp.* or *Lumbricus terrestris*): an acute (14 d) lethality test;an acute (72 h) sublethal test of avoidance responses; and a test for effects of prolonged (8 week) exposure on survival, reproduction, and growth. The developmental phase of this program, which has examined the performance of these three test methods using samples of field-collected and spiked soils contaminated with pesticides, metals, or petrochemical wastes, is nearing completion. Efforts to further standardize each test method are now underway, in conjunction with the preparation of a methodology document for soil toxicity tests using earthworms to be published and distributed by Environment Canada. A summary of the evolving procedures and conditions to be applied when performing each of these three biological test methods for measuring soil toxicity using earthworms is presented here.

Development of a Standardized Terrestrial Multispecies Test System for Risk Assessment. J.J. Scott-Fordsmand and P.H. Krogh. Department of Terrestrial Ecology, National Environmental Research Institute, Silkeborg, Denmark.

Risk assessment of chemical compounds is currently based on a compilation of single species toxicity-test data. However, in the field not only the direct stress effects on the species are important for the ecosystem but also the interactions between these species. In soils, this problem has only received little attention. Recently the problem has been encountered by performing toxicity tests with field collected intact soil columns, subsequently treated and *post hoc* analyzed for the species composition. Such systems, among others, lack the repeatability necessary in risk assessment and the results may be very dependent on actual soil type and species composition for each test. To eliminate this problem we introduce a standardized multispecies invertebrate test-system including the main biological components of the ecosystem in question. The exposure of the organisms is under well-characterized and standardized conditions and the initial species composition is known *a priori*. We describe the design of a multi-species system including an invertebrate species assemblage of enchytraeids and microarthropods closely resembling natural communities in agricultural soils. The test system contained a natural microbial activity. Using nonylphenol as an example we present the results of such a test and the statistical methods for the evaluation of this. The results obtained will be compared to the results from single species tests performed under similar conditions.

Soil Contamination from a Mercury Spill in The Andes Mountains: Environmental and Human Health Implications. C.D. Wren, ESG International, Guelph, ON.

The Minera Yanococha is located in northern Peru, and is the second largest gold producing mine in the world. Hg is naturally present in the ore and is refined as a side product of the gold. This year an estimated 42 tonnes of pure elemental Hg will be produced at the mine. The Hg is transported by truck overland to Lima approximately 600 km away. In June, 2000, one container became damaged during transport, and approximately 150 kg of Hg was spilled along numerous sections of highway. Residents from three local villages picked up the mercury and returned to their homes with it. Due to extensive handling and heating the Hg on stovetops over 200 people were exposed to toxic Hg

fumes, and several dozen people were hospitalized. Over 100 houses became contaminated, most with soil floors. During the next several weeks a full soil, road and house remediation program was implemented that involved over 150 workers. Medical experts and toxicologists were brought in from the United States. Environmental monitoring of soils, water, stream sediments, fish, crops and livestock is ongoing and the data will be used for human health and ecological risk assessment. More difficult to measure is the social impact to remote villagers that had their lives disrupted as a result of this incident.

Bringing Ecology Back into Ecotoxicology. P.M. Chapman. EVS Environment Consultants, North Vancouver, BC.

Differences between environmental toxicology and ecological toxicology (ecotoxicology) are reviewed in the context of real problems facing the Earth today. Environmental toxicology focuses on laboratory issues and testing costs; ecological toxicology focuses on ecological issues and the costs of an incorrect decision. General guidelines for acute and chronic testing are provided, as are ecotoxicological criteria for species selection (versus "standard" environmental toxicology criteria), laboratory versus field tests and mixed species testing. An example of the need for ecotoxicology is presented relative to estuarine sediments and their unique characteristics (overlying and interstitial salinity as a controlling factor, bioavailability measurements, benthos – "the paradox of brackish water" and seasonal, interstitial-salinity induced movements up- and down-stream). Current sediment toxicity tests, species used, end-points, problems and resolutions are reviewed. Most testing has involved single species, but community level toxicity tests are available. Specific recommendations are provided for ensuring estuarine sediments are evaluated based on ecotoxicology, not environmental toxicology. An overall framework based on ecological risk assessment is then proposed for combining ecology and toxicology to minimize uncertainty and maximize realism. Two alternatives are possible: extrinsic or intrinsic incorporation of ecology into toxicology (the latter is preferable). Final recommendations include: not separating the disciplines of ecology and toxicology; not relying on "snapshots in time"; developing and using tools to measure ecosystem status and indications of stress. The shift from environmental toxicology to ecotoxicology represents an important shift from reductionist to holistic approaches.

What is Wrong with the Sediment Quality Triad? U. Borgmann and W.P. Norwood. Environment Canada, National Water Research Institute, Burlington, ON.

The Sediment Quality Triad, consisting of measurements of: [1] chemical concentrations in sediments; [2] alterations in benthic community composition; and [3] sediment toxicity, constitutes the current "state of the art" in many assessments of sediment quality. We tested the adequacy of the Triad by addressing the four key questions identified by the federal/provincial/industry Aquatic Effects Technology Evaluation (AETE) program using data collected from lakes near Sudbury. The Triad successfully addressed two of these questions (Are contaminants getting in the system? and Is there a measurable response?). Triad data could not address the other two (Are contaminants bioavailable? and Are the contaminants causing this response?). Bioavailability can be determined by measuring contaminant accumulation in biota, but implication of cause is usually attempted mainly through correlations between the three components of the Triad. Sudbury area sediments are contaminated by many metals, but the concentrations of these metals are strongly correlated with one another. Effects correlate with numerous metals, making it impossible to identify cause from correlations of chemistry and effects. A complete assessment of sediment quality requires not just the Sediment Quality Triad components, both also a measure of contaminant bioavailability (e.g., bioaccumulation), and data on how much bioavailable contaminant is required to cause an effect (e.g., critical body concentrations).

What Can We Learn from Perplexing Sediment Quality Assessment Data? R.S. Carr[1] and M. Nipper[2]. [1]USGS, Marine Ecotoxicology Research Station, Corpus Christi, TX; [2]Texas A&M University-Corpus Christi, Center for Coastal Studies, Corpus Christi, TX.

From the numerous sediment quality assessment studies that have been conducted over the past decade, it is possible to show examples where there were excellent correlations between one or more sediment toxicity tests and the sediment contaminant concentrations and/or benthic communities structure data. There are also situations in which there are high concentrations of contaminants but little or no toxicity or vice versa. In the majority of studies in which solid-phase amphipod and sea urchin porewater toxicity tests have been conducted together, the porewater tests invariably are considerably more sensitive than the solid-phase tests. This is not surprising, since the urchin fertilization test is a highly sensitive sublethal method, whereas the amphipod test measures acute mortality only. There are examples, however, from highly contaminated sites where the sea urchin fertilization test was less sensitive than the amphipod test. Examples from Biscayne Bay, Boston Harbor, and other studies will be presented demonstrating such seemingly perplexing results with plausible explanations for and recommended interpretation of these types of data in light of some recent studies with nitroaromatic compounds. These perplexing data sets can provide clues as to the contaminants of concern and where additional studies need to focus.

Laboratory Studies of Confounding Factors Affecting Sediment Toxicity Tests and Integration of These Results in Interpretation of Field Studies. P.M. Jackman and K.G. Doe. Environment Canada, Moncton, NB.

Ammonia, sulfide and particle size distribution are several of the confounding factors that may contribute to an adverse response of a test organism in standard sediment toxicity tests. Knowledge of response of the different test species to these parameters is essential for selection of appropriate test species, as well as interpretation of toxicological data. Laboratory testing has been conducted to determine the level these confounding factors result in an adverse effect for several of the standard Environment Canada freshwater and marine toxicity test methods. These toxicity tests require characterization of sediment samples for parameters that may influence toxicity test results. Data from laboratory evaluations of ammonia, sulfide and grain size effects on uncontaminated combined with sediment characterization are integrated into the interpretation of toxic responses measured in field studies to evaluate if the response is due to natural causes or chemicals of concern (such as heavy metals or persistent organic pollutants).

Nickel Bioavailability and Toxicity to *Hyalella azteca* in the Presence of Whole and Fractioned DOM. L.E. Doig and K. Liber. Toxicology Centre, University of Saskatchewan, Saskatoon, SK.

Dissolved organic matter (DOM) has long been known to play a role in modifying metal bioavailability and hence metal toxicity to aquatic organisms. A series of Ni toxicity experiments were conducted as short-term water-only tests (i.e., in the absence of sediment) using DOM from various sources and fractions (Suwannee River humic and fulvic acids; whole peat, peat hydrophilic DOM (aqueous); and Little Bear Lake sediment pore water). Isolated DOM was added to reconstituted water in various amounts to evaluate effects on Ni bioavailability and toxicity to *Hyalella azteca* in 48 h water-only tests. It was found that with the addition of DOM (0-30 mg/L) there were no discernable trends in the 48 h LC_{50} data. Using a miniaturized ion exchange technique, it was found that the concentration of free Ni ion was only slightly decreased at the chosen test concentrations of Ni and DOM. While it is known that Ni will complex with organic matter, the test concentrations used were likely much greater than can be significantly complexed by the DOM. Therefore, any toxicity-modifying effect produced by the DOM may have been too subtle for our experiments to resolve. Overall, while DOM does not appear

to modify Ni toxicity at 48 h LC_{50} concentrations, DOM likely plays a much greater role in reducing Ni bioavailability at low metal concentrations where a greater proportion of the Ni is able to complex with DOM. Experiments to evaluate this hypothesis are currently underway.

Nickel Bioavailability and Toxicity Through a Sediment Core. W.P. Norwood[1] and U. Borgmann[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

Sediment cores from Richard Lake near Sudbury, Ontario, were sectioned, dated and analyzed for total metals. A strong Ni profile was observed in the sediment. This was compared to profiles of toxicity and bioavailability (bioaccumulation and overlying water concentration) to *Hyalella azteca*. The deepest sediment layers, deposited prior to industrial development, were non-toxic. Sediment toxicity was attributed to Ni dissolution into overlying water. Furthermore, differential bioavailability of Ni in surface and deeper sediment layers was observed. Based on Pb-210 dating and trends in Ni in the core, chronic toxicity of surface sediments from Richard Lake might approach non-toxic levels in about 15 years.

Ordnance Compounds in Marine Sediments: Are They a Concern? M. Nipper[1], R.S. Carr[2], J. Biedenbach[2], [illegible]. Hooten[2] and K. Miller[3]. [1]Texas A & M University – Corpus Christi, TX; [2]USGS, Marine Ecotoxicology Research Station, Corpus Christi, TX; [3]Naval Facilities Engineering Service Center, Port Hueneme, CA.

The biological hazard of ordnance compounds in marine environments was evaluated through toxicity testing with spiked sediments and pore waters. Tests were performed with 2,6-dinitrotoluene (2,6-DNT), tetryl and picric acid, which were individually spiked into a sandy and a muddy sediment with 0.1 and 1.1% total organic carbon (TOC), respectively. Experiments included 10 d amphipod solid-phase tests and porewater exposures analyzing sea urchin embryological development, macroalgae zoospore germination and polychaete reproduction. Some unique data were generated in addition to some expected results, such as higher toxicity in the sediment with lowest TOC, and higher toxicity in spiked seawater than in some porewater samples, which is likely due to binding of the chemicals to organic carbon in the pore water. Unexpected results included: a U-shaped toxicity curve for the muddy sediment spiked with picric acid; and higher porewater toxicity for samples from the spiked sediment with highest TOC than for filtered seawater. The role played by biodegradation products in these results was analyzed. It was concluded that in addition to identifying the hazard of the parent ordnance compounds, attention must be given to microbial degradation products and research is necessary to verify if such compounds will eventually be mineralized under natural conditions.

Endocrine Disruptors

Session Co-chairs: G.J. Van Der Kraak and M. Servos

Detailed Endocrine Assessment of Wild Fish Within the Northern Rivers Basins. M.E. McMaster[1], L. Peters[1], M.L. Hewitt[1], G.J. Van Der Kraak[2], K. Oakes[2], C.B. Portt[3] and N. Denslow[4]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Zoology, University of Guelph, Guelph, ON; [3]C. Portt and Associates, Guelph, ON; [4]University of Florida, Gainesville, FL.

We have initiated studies as part of the Northern Rivers Ecosystem Initiative (NREI) to examine reproductive endocrine function in wild fish downstream of three pulp and paper mills within the

Northern River Basins. These studies are utilizing all of the endocrine endpoints that we have developed over that last several years including examinations of gonadal development including histology (intersex), age to maturation, expression of secondary sex characteristics, circulating sex steroids, in vitro steroid productive capacity, gonadal apoptosis, circulating and pituitary gonadotropin levels, sex steroid binding proteins, hepatic and gonadal oxidative stress, circulating vitellogenin levels, hepatic estrogen receptors and gonadal androgen receptors. The first year of our study is complete with samples collected from fish downstream of both the large municipal sewage discharge from the town of Grande Prairie as well as downstream of the pulp mill located outside of Grande Prairie and two reference locations. Samples are now being analyzed and it is hoped that we will be able to separate out the effects of the two discharges on fish reproductive function. The study is funded through the NREI, the Toxic Substances Research Initiative and both Alberta Environment and Environment Canada.

Laboratory Exposures of *Fundulus heteroclitus* to Evaluate the Significance of Reverse Osmosis Treatment of Clean Condensates on the Endocrine Disruption Potential of BKPM Process Effluents. M.G. Dubé[1] and D.L. MacLatchy[2]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Department of Biology, University of New Brunswick, St. John, NB.

The objectives of this study were to identify, through laboratory exposures, a process stream from the pulping side of mill operations at a bleached kraft pulp mill (BKPM) which depressed steroid hormone levels in the mummichog (*Fundulus heteroclitus*), to determine the effectiveness of reverse osmosis (RO) treatment of condensates in removing the potential of mill process and final effluents to depress reproductive steroids in the mummichog, and to identify potential endocrine disrupting compounds (EDCs) in a BKPM process effluent. A 7 d and 21 d exposure were conducted where mummichog were exposed to RO feed effluent (1%, 3%), RO permeate (1%, 25%), combined mill effluent (CME) (1%, 5%, 50%), and final effluent (1%, 50%). Results showed that a specific condensate stream depressed plasma levels of testosterone in the mummichog and the responsible chemicals were not plant phytosterols or a component of weak black liquor entrainment of the condensate. In addition, our study showed that the potential of this condensate stream to depress reproductive steroids was removed by RO, which likely resulted in a final effluent that did not cause endocrine disruption to the mummichog at an environmentally-relevant effluent concentration (1%).

Increased Potential of Bleached Kraft Mill Effluent to Cause Endocrine Disruption in Fish Following Secondary Treatment. D.L. MacLatchy[1], M.G. Dubé[1,2], C.I. Gilman[1], A.K. Smitheram[1] and J.M. Culp[2]. [1]Department of Biology and Centre for Coastal Studies and Aquaculture, University of New Brunswick, Saint John, NB; [2]Environment Canada, National Water Research Institute, Environment Canada, Saskatoon, SK.

In August of 1999, mummichogs (*Fundulus heteroclitus*) were exposed in a portable artificial stream (mesocosm) system to 1% concentrations of primary and secondary treated effluents from a bleached kraft pulp mill (BKPM) in New Brunswick. Following a 21 d exposure, there were no differences among fish exposed to reference water or effluents in length, weight, condition factor or gonad size. However, male and female fish exposed to primary and secondary effluents had significantly decreased levels of plasma testosterone levels (secondary > depression than primary). These results add to the growing weight-of-evidence that although secondary treatment of BKPM effluent has reduced some measures of effluent toxicity (e.g., acute toxicity), concentrations of potential endocrine-disrupting compounds may be enhanced in treatment ponds. As well, this study also adds to previous work from our labs demonstrating the applicability of portable mesocosm use for studying endocrine disruption in fish exposed to effluents released into confounded, complicated estuarine environments.

Examination of Reproductive Function in Forage Fish Species Exposed to Natural and Anthropogenic Inputs from Monitoring the Aquatic Environment in the Athabasca Oil Sands, Alberta, Using Reproductive Responses in Small Forage Fish Species. G.R. Tetreault[1], M.E. McMaster[2], D.G. Dixon[1], W.N. Gibbons[3] and J.L. Parrott[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Golder Associates Ltd., Calgary, AB.

Fish were collected from the watershed surrounding the Athabasca Oil Sands deposit, including sites outside of the oil sands deposit, within the oil sands deposit and downstream of refinery waste waters. These fish are unexposed, exposed to naturally occurring oil sands related compounds, or exposed to natural as well as anthropogenic point source inputs, respectively. The main objective of this study was to determine whether reproductive parameters were altered in fish exposed to either the natural or anthropogenic sources relative to upstream reference fish. Field collections for reproductive assessment included sampling of resident fish for gonadal somatic indices (GSI), total fecundity and age as well as steroid production potential as measured using an *in vitro* gonadal incubation procedure. *In vitro* gonadal incubations have been used to assess sex steroid production in fish too small to obtain sufficient amounts of blood. Sites used in this study were often remote requiring the transportation of fish to a sampling location. Effects of this fish handling and confinement were evaluated to validate measurement of sex steroid production levels throughout the study. Whole body homogenates for sex steroid levels are also being assessed in attempts to develop methods useful in long term monitoring programs.

Free Radicals as a Possible Mechanism of Pulp Mill Effluent Induced Reproductive Dysfunction in White Sucker (*Catostomus commersoni*). K. Oakes[1], M.L. Hewitt[2], J.L. Parrott[2] C. Wood[3], L. Tremblay[4] and G.J. Van Der Kraak[1]. [1]Department of Zoology, University of Guelph, Guelph, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Nexfor Technology, Point Claire, QC; [4]Landcare Research, Lincoln, New Zealand.

Numerous studies over the past decade have identified impaired reproduction in feral white sucker and other fish species exposed to pulp mill effluents. We have conducted studies to evaluate the potential impact of free radical damage resulting from mill effluent exposure as a source of reproductive impairment. Feral white sucker (*Catostomus commersoni*) exposed to 50% bleached mill effluent (BME) for 4 d showed a modest increase in hepatic free radical damage (TBARS), but no increase in gonadal TBARS, nor any alteration in sex hormone profiles with exposure. Effluent from the same mill in 21 d lab exposures using immature rainbow trout (*Oncorhynchus mykiss*) resulted in a dose-dependant 30-90% increase in hepatic TBARS which was strongly correlated to an induction of mixed function oxidase (MFO) activity. Redox-active compounds could also contribute to lipid peroxidation as i.p. injections of ferric nitrilotriacetate (15 mg/kg body weight) resulted in significant increases in hepatic free radical damage and depressions in circulating steroid hormones. These findings suggest further investigation into the role of free radical damage as a mechanism of reproductive impairment in fish exposed to pulp mill effluent is warranted.

Is Coprostanol Estrogenic to the Freshwater Mussel, *Elliptio complanata*? F. Gagné[1], C. Blaise[1], H. Sabik[1], M.H. Salazar[2] and S.M. Salazar[2]. [1]Environment Canada, Saint-Lawrence Center, Montreal, QC; [2]Applied Biomonitoring, Kirkland, WA.

Coprostanol (5α-cholestane 3β-ol) is produced by the C5-reduction of cholesterol by fecal coliforms. This substance is found at high concentrations in urban effluents and nearby surface waters and sediments. In a previous study, freshwater mussels exposed in the field for 62 d in an urban plume were found to accumulate large quantities of coprostanol in their soft tissues (15 mg/kg dry weight).

Moreover, these mussels had elevated levels of Vn in the hemolymph which suggest estrogenic effects. Although municipal wastewaters are known to contain estrogens such as estradiol and nonylphenol able to induce vitellin synthesis in mussels, the estrogenic potential of coprostanol was investigated in freshwater mussels, *Elliptio complanata*. First, mussels were injected to increasing amounts of coprostanol and allowed to stand in aerated water for 72 h at 15°C. Afterwards, the levels of Vn in the hemolymph were assayed by the organic alkali-labile phosphate methodology. Second, a competitive estradiol binding assay was performed to measure the ability of coprostanol to compete in the binding of an estradiol analogue to cytosolic proteins of the gonad. Scatchard analysis of the binding data revealed that the receptors have a Kd between 0.5-1 nM for mussels which is comparable to rainbow trout receptors. Coprostanol partially reversed the binding of labeled estradiol-albumin to cytosolic proteins with an effective concentration that inhibits 50% of estradiol binding (EC_{50}) of 1 mM. Moreover, the relative molecular size protein-estradiol-albumin complex suggests that the protein receptor(s) have a molecular weight of at least 40 kda which is consistent with the reported molecular weights of avian and fish estrogen receptors. In addition, injection of mussels to coprostanol and estradiol leads to increased levels of vitellins in the hemolymph. The results suggest that coprostanol has some affinity to estradiol-binding sites (receptors) in the gonad and increases the levels of Vn in the hemolymph of mussels. The release of large amounts of coprostanol in surface waters by municipal effluent represents a potential threat to aquatic invertebrates. Possible mechanism of action of this substance will be discussed.

Effects of Water-borne 4-Nonylphenol and Estrogen on the Growth, Survival and Physiology of Atlantic Salmon (*Salmo salar*) Smolts. W.L. Fairchild[1], J.T. Arsenault[1], K. Haya[2], L.E. Burridge[2], J.G. Eales[3], D.L. MacLatchy[4], R.E. Evans[5], B.K. Burnison[6], J.P. Sherry[6], D.T. Bennie[6] and S.B. Brown[6]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]Department of Fisheries and Oceans, Biological Station, St. Andrew's, NB; [3]Department of Zoology, University of Manitoba, Winnipeg, MB; [4]Department of Biology, University of New Brunswick, St. John, NB; [5]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [6]Environment Canada, National Water Research Institute, Burlington, ON.

A recent study identified relationships between historical applications of an insecticide containing 4-nonylphenol (4-NP) and catch data for Atlantic salmon (*Salmo salar*) populations. To test the hypothesis that 4-NP impairs parr-smolt transformation, we exposed Atlantic salmon smolts to environmentally relevant, pulse doses of water-borne 4-NP (20 and 200 µg/L). We also exposed smolts to sustained doses of estradiol (E2) (100 and 300 ng/L). Osmoregulatory (plasma and tissue ions, gill ATPase), biochemical (glucose/glycogen), and endocrine (thyroid hormones, thyroid hormone deiodinases, vitellogenin, steroids, growth hormone and insulin-like growth factor 1) parameters were measured on smolts. Subsequent growth and survival in sea water was impaired in about 25% of fish from treatment groups (5% in control) in 1998. Although there was no apparent treatment related mortality in 1999, by October there were significantly more small fish in the 4-NP and E2 treatments. If the effects exerted by 4-NP are due to its estrogenic potential, then estrogenic activity stemming from other sources (e.g., domestic sewage, agricultural wastes or phytoestrogens from pulp mills) might influence present day salmon populations.

The Effects of Azamethiphos on Survival and Spawning Success in Female American Lobsters (*Homarus americanus*). L.E. Burridge, K. Haya and S.L. Waddy. Department of Fisheries and Oceans, Biological Station, St. Andrews, NB.

In southern New Brunswick, Canada, the salmon aquaculture has rapidly expanded to a $140 million industry. Recently the caged salmon have experienced infestations of sea lice. "Bath" treatments of the organophosphate pesticide, azamethiphos are used to treat the sea lice infestations. The period

of sea lice infestations are coincident with spawning of the American lobster (*Homarus americanus*). Salmon aquaculture sites and lobster nursery areas share the same water, resulting in a situation in which lobsters may be exposed to effluent from sea lice treatments. Preovigerous female American lobsters (n=72) were divided into two treatment and one control groups. Ovarian maturation and spawning was induced using elevated water temperature and long day length. Lobsters were exposed four times for one hour to either 10 or 5 µg/L of azamethiphos. These concentrations represent 10 and 5% of the recommended treatment concentration. Treatments were separated by two weeks and three experiments were conducted over three spawning seasons. Survival and success of spawning were monitored. Similar results were observed in the three experiments. For example, in the second experiment, only one lobster was dead after the third to 10 µg/L azamethiphos, but after the fourth exposure, 43% (10) of the lobsters had died. In contrast, only 8% (2) of those exposed to 5 µg/L died after the final treatment and there were no deaths amongst the controls. In a separate experiment the activity of acetylcholinesterase (AChE) in the muscle of exposed lobsters was measured. These data suggested a possible cumulative inhibition of this enzyme. Alternatively, increased sensitivity of the lobsters during the fourth treatment compared to earlier treatments may be related to seasonal differences in physiology or to the endocrine state of preovigerous and spawning females. Two months after the last treatment, spawning success, as assessed by the presence of extruded eggs, was also affected by exposure to the highest concentration of azamethiphos. Seven (54%) of the surviving lobsters exposed to 10 µg/L failed to spawn, while 2 (9%) of the surviving lobsters exposed to 5 µg/L of azamethiphos and only 1 (5%) of the control lobsters failed to spawn. Oocyte vittelin was resorbed by some of the lobsters in the azamethiphos treated groups.

Identification of Estrogenic Substances in Animal and Human Waste. B.K. Burnison[1], T. Neheli[1], D. Nuttley[1], A. Hartmann[2], R. McInnis[1], A. Jurkovic[1], K. Terry[1], T. Ternes[2]. H.-B. Lee[1], T.E. Peart[1] and M. Servos[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]ESWE Institute for Water Research and Water Technology, Wiesbaden, Germany.

A variety of estrogenic responses such as intersex and induction of vitellogenin have been identified in fish exposed to municipal effluents in Europe, the United States and Canada. Estrogenic compounds have been identified in municipal effluents and included industrial chemicals such as alkylphenols and Bisphenol-A, and natural estrogens such as 17ß-estradiol and estrone. The pattern and level of estrogenic chemicals detected in Canadian effluents are similar to those reported in other countries. Similar to human sewage it has been speculated that animal manures, that are applied to agricultural fields and may runoff into adjacent streams, may also contain a variety of estrogenic substances. Chemical fractionation of hog manure samples using the toxicity identification and evaluation (TIE) approach, isolated several natural hormones as well as the compound equol, which is a metabolite of certain phytoestrogens. The relative potency of equol was confirmed to be three orders of magnitude less than 17β-estradiol and the chemical determination of equol was shown to be approximately 6 mg/L in the hog manure (700 sow farm). Subsequent field studies have confirmed that application of manure to a field can result in the runoff of estrogenic compounds, including equol. However, the biological significance of a short-term, low-level pulse of these compounds is not known.

Effects of a Potent Estrogen Mimic on Aquatic Populations: A Whole-Lake Addition Experiment. K. Kidd. Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Significant evidence exists that aquatic organisms are being adversely impacted by chemicals that mimic natural hormones. However, it is not known whether the responses observed at the organism level are indicative of impacts at higher levels of biological organisation. To investigate this unknown and to calibrate and validate the responses between individual- and population-level effects, we are conducting a multi-year study at the Experimental Lakes Area (ELA) in northwestern Ontario. The

potent estrogen mimic 17α-ethynylestradiol (EE2) used in birth control pills is found at significant concentrations in sewage effluents. This estrogen is known to induce vitellogenin (an egg protein precursor) production in fish downstream of these effluents, and impact upon gonadal development and reproductive behaviour in male fish. In this study we will be examining the effects of a continuous whole-lake addition of EE2 (beginning in 2001) on organisms across all trophic levels. In preparation, two years of baseline population monitoring and enclosure experiments have been conducted on lakes at the ELA in 1999 and 2000. Microbial, algal, zooplankton, benthic invertebrate and tadpole populations are characterized in the study and reference lakes to examine the effects of EE2 on lower-trophic-level organisms. Population sizes, age to maturity, fecundity, spawning behaviour, gonadal development and several biochemical parameters are being monitored in four fish species for contrast before and after addition of this estrogen mimic. Results from this study will be critical in understanding the magnitude of impacts of hormone mimics on aquatic organisms and for interpreting the ecological relevance of field and laboratory assessment studies.

Fathead Minnow Lifecycle Exposure to Ethinyl Estradiol. J.L. Parrott, G.G. Fodor and B.R. Blunt. Environment Canada, National Water Research Institute, Burlington, ON

As part of their response to the endocrine disruption issue, USEPA and OECD have launched programs to assess various fish bioassays for ability to screen for endocrine disrupting substances (EDS). Tests include short-term exposures as initial EDS screens, and long-term or lifecycle exposures of various fish species for higher testing tiers. The goal of this study was to assess the use of fathead minnow full lifecycle assay for detection of EDS, and to see whether shorter exposures (8, 15, 30 or 60 d) would produce responses that could be linked to longer-term reproductive responses in the adult fish through 150 d. Fathead minnow eggs were exposed to 0, 0.32, 1, 3.2, 10, 32 ng/L ethinyl estradiol (EE2) in a flow-through exposure system. Fish were followed through larval, juvenile and adult stage. Effects on growth secondary sex characteristics, physiological alterations (changes in liver size, gonad size, fecundity) and reproductive biomarkers (ability of the adult fish ovaries/testes to produce sex hormones) were examined at several exposure times. No statistically significant changes were seen in eggs (hatch, mortality) fry at 8 or 15 d (length, weight, mortality) or juveniles up to 30 d post-hatch. At 60 d, fish were significantly smaller (32 ng/L). Secondary sex characteristic (measured as ovipositor index) was the most sensitive measure of effect after 60 d in fathead minnows exposed to >3.2 ng EE2/L. Following the fish through maturation, reproduction, and sampling at 150 d provided several endpoints that responded to ethinyl estradiol. The most sensitive endpoint was fertilization success (affected at 0.32 ng/L), followed by male secondary sex characteristics (1.0 ng/L). GSI, ovipositor index and sex ratio (the three highest concentrations contained no male fish) showed changes at 3.2 ng/L in 150 d old fish. The results show that although there are some endpoints that show changes after 60 d, the endpoints measured at 150 d are more sensitive to the effects of ethinyl estradiol. From these results it appears that shortening the test to 60 d is possible, but may result in a ten-fold loss of sensitivity.

Influence of Estrogenic Contaminants on Amphibian Sex Differentiation. C.A. Mackenzie[1], C.D. Metcalfe[1], M. Berrill[1] and B.D. Pauli[2]. [1]Watershed Ecosystems Program, Trent University, Peterborough, ON; [2]Environment Canada, Canadian Wildlife Service, Hull, QC.

Despite continued concerns over declining world amphibian populations, few studies have addressed the effects of environmentally relevant contaminants on the sexual differentiation of anurans. Leopard frog (*Rana pipiens*) tadpoles were exposed to aqueous solutions of model estrogenic and anti-estrogenic compounds during their larval development. Results indicate that *R. pipiens* are sensitive to low concentrations of estradiol, with 1 and 10 µg/L treatments producing increased incidence of females and ovotestes, an intersex condition with both ovarian and testicular tissue present in the

gonad. Estradiol treatments at 50 and 100 µg/L produced 100% females. Exposure to ethinyl estradiol (1 and 10 µg/L) was effective at increasing the proportion of females and ovotestes. A significant increase in ovotestes also resulted from exposure to nonylphenol (10 and 100 µg/L), a degradation product of the nonylphenol polyethoxylate class of nonionic surfactants, flavone, a flavonoid aromatase inhibitor (10 and 100 µg/L), and an anti-estrogen (ICI 182780) (1 and 10 µg/L). These results indicate that amphibians are sensitive to low concentrations of endocrine disrupting chemicals (EDCs) found in aquatic environments. Amphibians may be exposed to EDCs from agricultural run-off of animal manure containing estrogens, from sewage treatment plant effluent containing alkylphenols and steroids, as well as from pulp mill effluent containing natural flavonoid aromatase-inhibitors. These results shed some light on the potential implications of current water management practices on wildlife health.

Mechanisms of Action of Polycyclic Aromatic Hydrocarbon (PAH)-Induced Testosterone Production in Male Fish. M. Evanson and G.J. Van Der Kraak. Department of Zoology, University of Guelph, Guelph, ON.

PAHs (polycyclic aromatic hydrocarbons) are ubiquitous, aquatic contaminants and have been identified as major risk factors for inhibited ovarian development and disruption of endocrine function in female fish. The present study examines the effects of selected PAHs (β-naphthoflavone, naphthalene, retene) on testosterone (T) production in male goldfish (*Carassius auratus*). All PAHs tested (0.01-100 µM) had no effect on their own, but potentiated gonadotropin- (human chorionic gonadotropin- hCG) and prostaglandin- (PGE_2) stimulated testosterone production by testis fragments *in vitro* in a dose- and time-dependent manner. Peak testosterone levels occurred at the median PAH doses (0.1 and 1.0 µM). Similar trends were observed in rainbow trout (*Oncorhynchus mykiss*) where β-naphthoflavone enhanced salmon gonadotropin-induced T production. *In vivo* β-naphthoflavone exposures in goldfish (2 intraperitoneal injection over 4 d; 0, 10-20, 50-70, and 70-100 mg/kg) resulted in a significant, 3-fold increase in plasma T at the 50-70 mg/kg dose. The putative role of the gonadotropin-adenylate cyclase signal transduction pathway in the observed potentiation of testicular androgen production was subsequently investigated. β-naphthoflavone did not potentiate forskolin (adenylate cyclase activator)- or 8-bromo-cAMP-induced T production. β-naphthoflavone also did not enhance the conversion of 25-hydroxycholesterol or the metabolism of pregnenolone, to testosterone. These studies demonstrate that PAHs, while inactive on their own, enhance fish steroid biosynthesis and that these actions may be modulated by a route other than the gonadotropin-adenylate cyclase pathway.

The "Estrogenicity" of Black Liquor and a Putative Environmental Estrogen in Rainbow Trout Hepatocytes. J.P. Sherry, T. Hooey, N. Spitale and M. Kohli. Environment Canada, National Water Research Institute, Burlington, ON.

An *in vitro* bioassay based on the induction of the egg yolk protein vitellogenin (Vg) in primary cultures of rainbow trout hepatocytes was used to test black liquor from the pulping process for estrogenicity. The black liquor induced Vg in the cultured hepatocytes. The sensitivity of the hepatocyte based assay to black liquor preparation will be compared to other *in vitro* test systems. The black liquor, however, did not induce detectable amounts of Vg in whole fish bioassays based on *i.p.* and waterborne exposure routes. We will also report on the ability of a putative environmental estrogen to induce Vg in trout hepatocytes. That compound has been detected in pulp mill effluent and is reputed to be estrogenic in fish.

Establishing a National Agenda on the Scientific Assessment of Endocrine Disrupting

Substances in the Canadian Environment. M. Servos[1] and M. Wade[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Health Canada, Health Protection Branch, Ottawa, ON. (Co-chairs of the 5-NR EDS Working Group).

The Endocrine Disrupting Substances (EDS) issue is broad in scope and, as a result, is linked directly to numerous other national environmental health issues and initiatives. Its complexity will necessitate a coordinated response from federal government departments, industry, academia and other organizations. Related activities in other jurisdictions, especially the United States and Europe, will influence development of public opinion and policy in Canada over the next few months and years. The 5-NR EDS Working Group recently completed a National Agenda for the scientific assessment of endocrine disrupting substances in the Canadian environment. This federal strategy for addressing this issue, developed primarily through a multi-department, multi-stakeholder workshop and consultation, will be presented and discussed. To address the issue effectively it is recommended that the federal government focus on four major areas: [1] national leadership and communication on the EDS issue; [2] establishing a better knowledge base of the exposure and effects of EDS in the Canadian environment; [3] national and international harmonization of screening and testing protocols; and [4] enhanced scientific assessment and action on priority substances. Each of these areas will be discussed, including research priorities identified.

Pulp and Paper Environmental Effects Monitoring

Session Co-chairs: I. Cloutier and R. Hubbard

Review of Cycle 2 Fish Studies from the Pulp and Paper EEM. A. McGeachy[1], K.R. Munkittrick[1,2], M.E. McMaster[2] and S.C. Courtenay[3]. [1]Department of Biology, University of New Brunswick, Fredericton, NB; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB.

Cycle 2 pulp and paper EEM reported April 1, 2000, and preliminary analysis of the fish survey results have been conducted. In Cycle 1 of the pulp and paper EEM, the fish survey had been poorly done with only 8% of the studies successful based on numbers of fish caught; 53% were unsuccessful. A number of generic problems were identified, and significant changes were made to the program to try and refine the approach. In freshwater, the dominant problems in Cycle 1 were related to an inability to catch fish, uncertain exposure, problems with reference sites, the presence of confounding factors and incomplete or poor reporting of data. Results, problems encountered and lessons learned will be compared for Cycle 1 and 2 data. In Cycle 2, the success of marine and estuarine surveys was low, and these studies continued to present challenges in Cycle 2. A variety of alternatives were used in Cycle 2, including on-site mesocosms and the success of these applications will be reviewed. A summary of research needs will be presented, as will recommendations for Cycle 3 pulp and paper. New developments in the program reflect some of the adaptations developed for metal mining EEM, including: [1] setting alpha and beta equal, and its consequences for study design and interpretation; [2] discussion of the role of effect size in power analysis and study design; and [3] non-lethal sampling protocols for EEM.

Quantifying Impacts of Pulp Mill Effluent on Fish in Canadian Marine and Estuarine Environments: Problems and Progress. S.C. Courtenay[1], H.M.C. Dupuis[1], W.R. Parker[2] and A. Colodey[3]. [1]Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [2]Environment Canada, Environmental Protection Branch, Fredericton, NB; [3]Environment Canada, Environmental Protection Branch, Vancouver, BC.

Amendments to the federal *Pulp and Paper Effluent Regulations* (PPER) in 1992 required all mills in Canada to conduct an Environmental Effects Monitoring (EEM) Program on three year cycles beginning in 1993. As one component the EEM, mills were required to compare indices of growth, survival and reproduction in wild-caught fish exposed to effluent (near-field) and not exposed to effluent (reference). A review of Fish Surveys carried out in the first cycle concluded that they contributed useful data in the freshwater environments for which they were designed, but generally performed poorly in the more complex marine and estuarine environments. Problems encountered were discussed at a national workshop in 1997 and a number of refinements and alternative approaches were recommended for the second cycle. As a result, more sedentary species were selected as sentinels in Cycle 2, pilot studies were carried out with caged bivalves and mesocosms (on-site bioassays) and more attention was paid to the movement of water and distribution of effluent. The degree to which these measures resulted in more useful data being contributed by Fish Surveys will be discussed in this talk and remaining challenges will be identified.

The Adult Fish Survey for EEM - Is it Worth it? Examples from Western Pulp Mills. M.D. Spafford[1] and B.M. Shelast[2]. [1]Alberta Pacific Forest Ind. Inc., Boyle, AB; [2]Stantec Consulting Ltd., Calgary, AB.

In 1998, five Alberta Pulp mills completed EEM Cycle 2 adult fish surveys on the Athabasca River. Many fish were sacrificed to obtain sample sizes of 25 per sex per species per site in Cycle 1 and a sample size of 40 in Cycle 2. Power analysis conducted on both data sets indicated that a sample size of 25 or 40 was not sufficient to detect any but a large difference (or effect). The effort to conduct the survey was at least 14 d for all mills. A common problem was the depletion of adult fish in reference and exposure areas in the latter stages of the surveys. The adult fish survey method in its current form can only detect drastic effects and the survey itself is far more damaging to fisheries than the mills that are being monitored. Alternatives have been tested and indicate more sensitivity with community level approaches without the large amount of sacrificed fish and half the current effort. Alignment with the Metal Mining EEM program is also suggested as an alternative to low impact mills.

The Five-Mill Athabasca River EEM Study – A Basin Approach to Monitoring. B.M. Shelast[1], M.E. Luoma[1] and C.G.J. Fraikin[2]. [1]Stantec Consulting Ltd., Calgary, AB; [2]Golder Associates Ltd., Calgary AB.

All five pulp and paper mills located within the Athabasca River Basin jointly undertook a study for the Cycle 2 Environmental Effects Monitoring (EEM) program. The primary objective was to examine whether pulp mill effects were taking place on a local (i.e., mill-specific) scale. A secondary objective was to determine whether pulp mill-related effects were occurring on a cumulative or more progressive scale as one moved downstream in the Athabasca River Basin. Temporal trends also were investigated as part of these objectives. The study afforded an opportunity to place a local-scale event into a basin-wide context, thereby facilitating a more ecologically meaningful interpretation of any effect. Although there were detectable effects on a local scale, there was no clear response pattern. No significant changes in the adult fish population and benthic invertebrate community were observed at a regional level.

Comparing Results from White Sucker (*Catostomus commersoni*) and Iowa Darter (*Etheostoma exile*) in a Pulp Mill EEM Study: The Relative Merits of Long-Lived, Roaming Versus Short-Lived, Sedentary Sentinel Species. C.B. Portt[1], G. Coker[1], B.W. Kilgour[2] and I. Cloutier[3]. [1]C. Portt and Associates, Guelph, ON; [2]Water Systems Analysts Inc., Guelph, ON; [3]Abitibi-Consolidated Inc., Montréal, QC.

The population characteristics of white sucker(*Catostomus commersoni*) collected from one exposed reach and one reference reach of the Winnipeg River were virtually identical. The population characteristics of Iowa darters (*Etheostoma exile*) collected from two locations within the same reference reach differed in several respects. Darters from one reference location were similar to those from the exposed site, while those from the second reference location were quite different. Roaming species integrate the habitat conditions over a larger area than sedentary species, which reflect site-specific conditions. Site-specificity necessitates multiple sampling locations, but facilitates more complex sampling designs, such as gradient designs. The ability to utilize more complex sampling designs could be extremely useful where upstream reference sites are unavailable or multiple discharges occur. Knowledge of how various habitat characteristics influence the population characteristics of sedentary sentinel species is required to improve interpretation of results.

Field Studies Examining Reproductive Function in Wild Fish Downstream of the Bowater Newsprint Mill in Gatineau, Quebec. M.E. McMaster[1], G.R. Tetreault[1], K.R. Munkittrick[1,2], C. Cobden[3] and M. Groves[3]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Biology, University of New Brunswick, Fredericton, NB; [3]Bowater, Gatineau, QC.

Bowater Pulp and Paper Canada Inc - Gatineau newsprint mill and Environment Canada undertook an alternative fish research study in order to clarify and validate the findings of Cycle 1 Environmental Effects Monitoring (EEM) results. In addition, objectives of the research study included an expansion of field collections including other fish species, studies to de-couple confounding factors, an evaluation of the appropriateness of using less mobile forage fish in the EEM program, and an examination of the appropriateness of the initial reference area selection in Cycle 1 by expanding on the number of reference sites used. Although our attempts to collect white sucker (used in Cycle 1) were not successful, our 1998 and 1999 collections of yellow perch failed to demonstrate any significant site differences. A more in depth reproductive evaluation also failed to demonstrate alterations in circulating steroid levels in yellow perch downstream of the effluent discharge. In depth studies with the forage fish, johnny darter, demonstrated that these species might be less mobile than some of the larger species used during Cycle 1 of the EEM program. Female johnny darter downstream of the effluent discharge demonstrated increased growth and increased investment into reproductive development, although gonadal tissue demonstrated some reductions in steroid production potential relative to the reference fish.

A Pickle Jar is a Pickle Jar and a Fish is a Moving Target but a Fish is not a Fat Bag and Bivalves Don't Eat Pickles. M.H. Salazar and S.M. Salazar, Applied Biomonitoring, Kirkland, WA.

Bivalves have been used to estimate chemical bioavailability and biological effects in laboratory and field exposures for over 30 years because they are good surrogate test organisms. Bivalve tests have been conducted with static and flow-through systems in the laboratory, mesocosms in the field, and using *in-situ* cages. We have conducted tests under all of these exposure conditions and have learned a great deal about the advantages and pitfalls of each approach. It appears that any test chamber may adversely affect their health because bivalves are sensitive to levels of both suspended particulate matter as a food source and adequate flow to deliver that food. This observation has important implications for environmental effects monitoring (EEM) at pulp and paper mills since fish mesocosms and caged bivalves have been proposed as alternatives to the adult fish survey. The shift from pickle jars to mesocosms to field studies is not really about measuring effects. It is about providing environmentally realistic test exposures. This paper will discuss our work on characterizing exposure and effects in bivalves and compare results from various exposure systems. For purposes of this discussion, a "pickle jar" will refer to any enclosed test chamber.

Overestimating Toxicity

The primary effect of laboratory-induced stress is reduced growth, and this is usually attributable to nutritive stress. Bivalves in laboratory tanks seldom, if ever, achieve the growth rates measured in natural populations or in field-deployed mussels. One example of this comparison from our work is shown in Fig. 1. Mussels of a similar initial size were transplanted to six field stations and held in the flow-though laboratory tanks. Lab mussels in flow-through tanks had significantly lower whole-animal wet-weights, tissue weights, and shell lengths after a 12 week exposure. This particular site was selected for the trailer with flow-through tanks because it had the lowest concentrations of chemicals in seawater and the highest growth rates ever measured in field-transplanted mussels. Our studies also suggest that laboratory exposures tend to overestimate the concentration at which effects begin to occur. A comparison of mussel growth rates measured in the lab and in mesocosms with mussel growth rates measured from *in-situ* exposures at various concentrations of tributyltin (TBT) is provided in Fig. 2. Growth rates increased exponentially for mussels caged *in-situ* mussels, but linearly for mussels held in the lab and in mesocosms. Over-estimating toxicity in the lab and in mesocosms has significant ramifications for effects monitoring and in the development of national water quality criteria in the US and Canada.

Bridging the Lab-Field Gap

Fish mesocosms and caged bivalves are being proposed as alternatives to the adult fish survey for

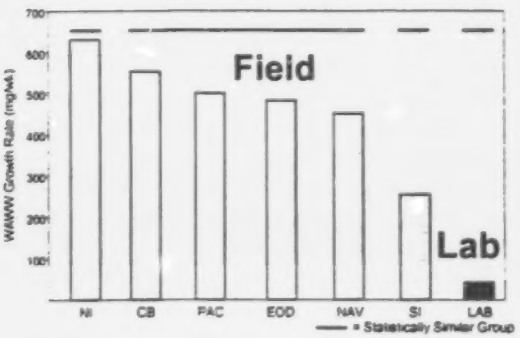

Figure 1. Lab & field mussel growth.

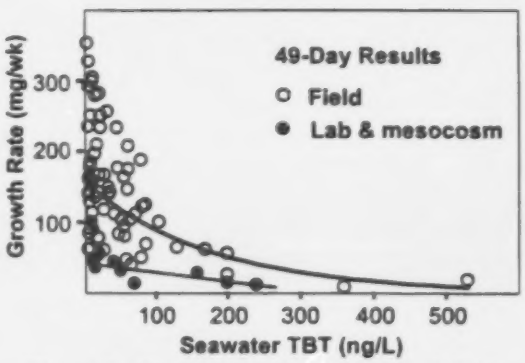

Figure 2. Growth in lab, mesocosm, & field.

EEM, at least in part, because they potentially combine elements of experimental control characteristic of laboratory exposures with elements of environmental realism from traditional field monitoring (Fig. 3). Lab tests, mesocosms, and caged bivalves all facilitate conducting experiments rather than just making observations. Fish surveys, wild bivalve surveys, and benthic community studies are more observational rather than experimental approaches. There is a continuum of increasing environmental realism and a continuum of experimental control and each method must be validated based on other observations. For example, we found that weekly measurements of field- transplanted mussels reduced growth rates and switched to bi-weekly measurements and found no significant difference in growth rate when compared with growth rates of adjacent wild mussels.

Mesocosms versus Caged Bivalves

We have compared mussel growth in mesocosm control and treatment tanks with growth in caged mussels placed adjacent to the seawater intake (Fig. 4). It was difficult to identify differences among growth rates for mussels in the mesocosm tanks, even though field studies indicated there should be effects. The pier control mussels grew significantly faster than the tank control mussels. The

mesocosm system included a high-volume pump to maximize flow rates and potential food sources, but a large percentage of the particulate material became trapped in the pipes and was not available to test animals. It is important to validate results by using field controls to test for potential differences in exposure and effects in all test systems.

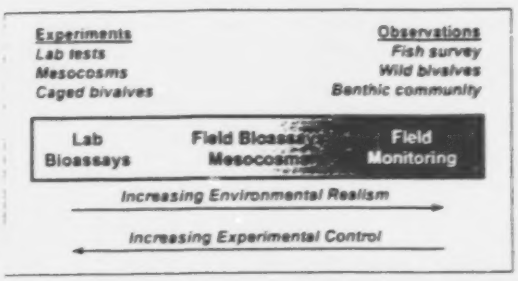

Figure 3. Bridging the lab-field gap.

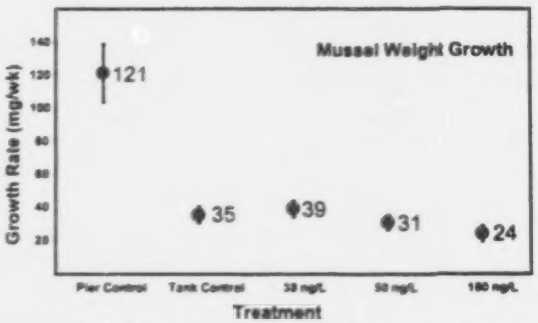

Figure 4. Pier control versus mesocosm tanks.

Need for Field Validation

Field validation for mesocosm systems can help explain observed results. In a recent mesocosm study to assess pulp mill effluents, control mussels had about 40% less lipids than mussels prior to placement in mesocosm tanks (Fig. 5). As with our mesocosm tests, there was not much difference in end-of-test lipid content among mesocosm treatments. This made it difficult to identify all potentially significant effects associated with exposure to mill effluents. Significant declines in lipids and increase in percent water are indicators of stress, and the use of stressed mussels most likely lead to an overestimation of toxicity and an underestimation of bioaccumulation potential. This adds a great deal of uncertainty to the test results. As suggested by the authors, the 40% loss of lipids during the test could have been associated with spawning. However, if the mussels spawned, it is likely that mill-related chemicals accumulated during the test were eliminated with the spawning event(s). It is unclear if and when the mussels spawned during the 89 d exposure period. The very low lipid content

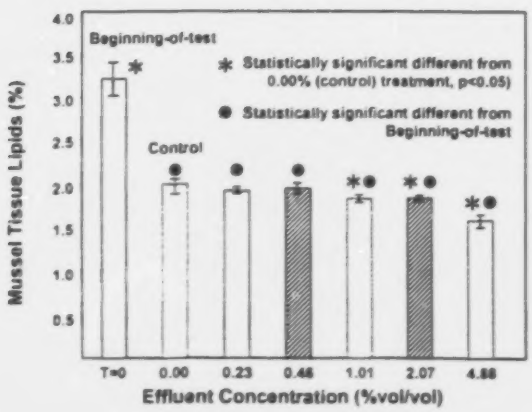

Figure 5. Mesocosm-induced stress.

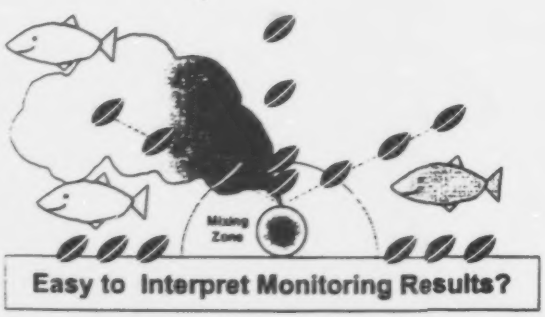

Figure 6. Fish are moving targets.

suggests that they either spawned immediately before ending the test, or they spawned earlier and, due to insufficient food in the mesocosm tanks, could not fully recover by the end of the study. As with most other mesocosm studies conducted for EEM, field validation was not conducted. Without determining the effects of containment, overcrowding, flow rates, and other variables on test animals, it is inappropriate to draw conclusions regarding the ability of this on-site system to provide environmentally realistic test conditions, use results from this test to justify future use of this approach, or use these data for regulatory purposes.

Moving Targets

Fish are moving targets. This makes it difficult to collect them, to satisfy monitoring requirements, and to interpret results. Some of the fundamental concerns are: [1] has exposure occurred? [2] if so, was it at the site of interest? [3] how long was the exposure? and [4] has the fish been exposed to other chemicals? The use of caged bivalves for effects monitoring eliminates these concerns because of the experimental control inherent in the approach (Fig. 6). Caged bivalves can be strategically placed along suspected chemical gradients and results validated by measuring natural populations in the vicinity.

One significant area that has not been adequately addressed in terms of laboratory toxicity tests, ecological risk assessments, or EEM is the importance of threatened and endangered species. In the US, freshwater bivalves are the most imperiled animal group. Freshwater bivalve populations in the US are the most diverse in the world, but their densities are decreasing due to a number of factors such as dredging, filling, diking, decline of fish hosts, and chemical contamination. Freshwater bivalves are particularly susceptible to environmental stresses. Not only can chemicals directly affect freshwater bivalves, but indirectly because they rely on fish as a host for the glochidial stage. Therefore, a decline in fish species may also affect the survival of individual bivalves and ultimately bivalve communities and populations. It has been suggested that the adult fish survey may have more of an impact on adult fish populations than the mill effluent. The potential impact of adult fish surveys impacting threatened and endangered freshwater bivalves in Canada has not been addressed.

Table 1 provides a comparison of caged bivalves, resident bivalves, mesocosm bivalves, and the adult fish survey for EEM applications. This comparison uses many of the criteria developed by the EEM program. It includes the number of animals available for testing, time necessary to collect those animals, time to conduct the test, cost of the test, the ability to verify exposure, mapping ability,

Table 1. Comparison of the adult fish survey with bivalves: caged, resident, and in mesocosms.

	Caged Bivalves	Fish	Resident Bivalves	Mesocosom Bivalves
Number Animals	3000	50	300	3000
Exposure	verified	no	?	verified
Experiment	yes	no	no	yes
Mapping	yes	no	?	no
Sediment	yes	?	yes	?
Defensible	yes	?	?	?
Efficient	yes	?	yes	no
Flexible	yes	?	no	?
Program	yes	?	?	?
Collection Time	hours	weeks	hours/days	hours
Cost	low	high	low	high
Ease of Interpretation	yes	no	no	yes?

associations with sediment, scientific defensibility, efficiency, flexibility, and programmatic applications. We believe that in each category, caged bivalves are equal to or superior to the other approaches. This is evident in the most important category, "ease of interpretation." In ecological risk assessments, characterization of exposure and characterization of effects are the two most important monitoring elements. They may also be the most important to EEM. If exposure is unclear, measured effects are of limited value.

Surveys of natural bivalve populations are probably most similar to the adult fish survey in terms of environmental realism, number of test animals normally used, lack of experimental control, and questionable exposure. Exposure is questionable because there are no direct measurements of exposure and it is not clear if the effects endpoints represent the last day, the last week, or the last month. This is even more important when the organic enrichment associated with some effluents can increase growth rates. Therefore, it would be misleading to assume that faster growth rates are necessarily associated with healthier test animals. There is also a high degree of uncertainty with estimates of age and shell growth. Given the extreme variation in size in most wild bivalve studies and the imprecision in the measurements, it is unlikely that they will ever approach the discriminating power of the caged bivalve methodology. This is probably why national and regional mussel watch programs have not been more successful.

Underestimating Exposure

One important reason for using bivalves is that they integrate chemical exposure by accumulating and concentrating chemicals in their tissues. This includes both dissolved and particulate pathways (i.e., water and food). Holding bivalves in cages in-situ does not appear to alter exposure pathways or growth. However, holding bivalves in test systems consisting of tanks and pipes may not adequately simulate all dietary pathways, as our mesocosm work has shown in some environments. Dietary pathways have been shown to be important for fish in some pulp and paper environments. Caging fish may also affect dietary exposure pathway, particularly if bottom feeding fish do not consume contaminated food or if they are fed uncontaminated food. Fish may be less sensitive to water flow and suspended particulate matter. However, if fish mesocosms alter dietary exposure pathways by excluding contaminated food, they may overestimate toxicity and underestimate exposure as our bivalve mesocosms did. A field control is needed to validate exposure and effects. Fat bags (semi-permeable membrane devices) are not good surrogates for fish because fat bags do not adequately represent total chemical exposure. Fat bags only estimate water pathways of exposure and do not accumulate chemicals bound to suspended particulate matter. Measuring tissue concentrations of chemicals in organisms remains the most environmentally realistic method for characterizing chemical exposure, and several studies have shown that bivalves can be used as a reasonable surrogate for fish.

Caged Bivalves for Biomonitoring, Mapping, and Source Identification

Caged bivalves can be used for source identification and to delineate the extent of chemical contamination in 3-dimensional space and time (Fig. 7). They can be used to quantify exposure and effects over space and time by measuring chemicals accumulated in their tissues and associated biological effects such as growth and reproduction. Results can validated by measuring natural populations in the vicinity.

Bioaccumulation is the link between environment and organism and should be emphasized more in EEM. It is not sufficient just to know if chemicals are present by taking discrete water samples that only evaluate an instant in time. Biological integrators like mussels are necessary to evaluate whether these chemicals are biologically available and likely to cause effects. Bivalves are particularly well suited to quantifying exposure because of their ability to integrate chemicals of concern or chemical tracers in their tissues as they filter the water for food. Fig. 8 shows how tissue chemistry can be used as a focal point to establish links between external exposure from water and sediment in field studies

using caged bivalves and from studies of traditional benthic community structure. Laboratory approaches can include both bioassays and mesocosm studies of benthic community structure.

Recently, we have developed and exposure-dose-response (EDR) triad (Fig. 9) that is more consistent with the ecological risk assessment paradigm of characterizing exposure and effects than other approaches. Our approach was refined using elements of the sediment quality triad, the exposure-uptake-effects triad, and developments in ecological risk assessment. Recently, several investigators have questioned the use of the sediment quality triad. At this Aquatic Toxicity Workshop, it was suggested to add bioaccumulation as a necessary component, thus creating a quad. We prefer the EDR triad because it emphasizes the importance of bioaccumulation as a focal point, and a link

Figure 7. Exposure & effects over space & time.

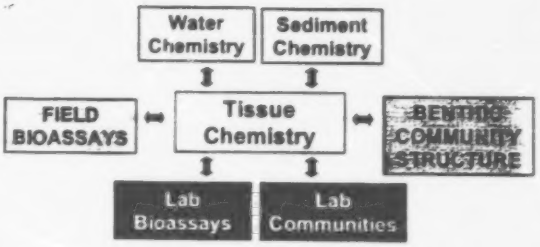

Figure 8. Tissue chemistry links.

between other monitoring approaches (Figs. 8, 9). We need to change the way we think about environmental assessments to focus on quantifying exposure first and then focus on the effects. This is a major shortcoming of emphasizing effects-based approaches in EEM. If exposure is not adequately characterized, the effects measurements are less powerful indicators. EEM should consider a risk assessment-based approach, not an effects-based approach.

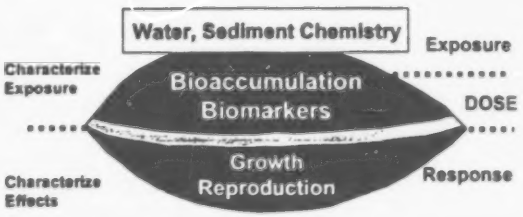

Figure 9. Exposure-dose-response triad.

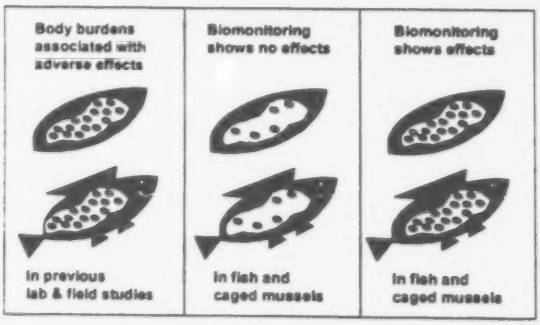
Body burdens associated with adverse effects
Biomonitoring shows no effects
Biomonitoring shows effects
In previous lab & field studies
In fish and caged mussels
In fish and caged mussels

Figure 10. Proposed monitoring framework.

Once relationships have been established between water and sediment chemistry, tissue chemistry, and effects, it will be possible to establish effects thresholds based on body burdens. Fig. 10 shows how body burdens can be used to establish numerical criteria where effects are expected. This graphic is intended to show a practical approach to utilizing relationships established between tissue chemistry and adverse biological effects in previous studies to develop a threshold effects level for predicting effects from biomonitoring data. In the first column, a threshold effects level is established from tissue concentrations of a given chemical (indicated by the dots) associated with effects as

measured in laboratory and field studies using fish and bivalves. The relative concentration of the chemical is indicated by the relative number of dots. In the second column, biomonitoring of tissue burdens indicates no expected effects because the tissue concentrations are below the predicted thresholds, as shown by a fewer number of dots. In the third column, biomonitoring of tissue burdens indicates expected effects because the tissue concentrations are above predicted thresholds, as shown by an equivalent number of dots. This approach uses the bioaccumulation link between exposure and effects to establish effects thresholds and could be used as part of EEM as a regulatory framework.

Integration & Synthesis
We have refined and standardized the caged bivalve methodology for various species and habitats. The application of this methodology has been enhanced by cooperative studies with our colleagues from Environment Canada in Montreal who have added a suite of biomarkers. These biomarkers can be used to help explain the relationship between exposure, dose, and response and to help establish relationships between bivalves and fish. The EDR triad has been developed as a framework for applying these results. During this development process, pitfalls associated with on identified and a method established for validating results from these other approaches through testable hypotheses. A pickle jar will always be a pickle jar and may not simulate natural conditions very well, even if it is a flow-through pickle jar and located on site. The use of field controls can help validate results from those exposures. Fish will always be moving targets, but can also be used as part of an EDR triad and as part of an integrated approach that includes controlled experiments in the field. Fat bags do not adequately simulate natural chemical exposures to fish, particularly when dietary exposure is important. Bivalves don't eat pickles but they do eat suspended particulate matter and it is important to include and quantify food in all pickle jars. At the 1998 ATW, Peter Campbell gave an inspirational plenary presentation on ecotoxicological challenges for the next century that included the following messages: [1] toxicity test results are tenuous; [2] get out of the lab and into the field; [3] don't just whine about limitations of field work, do something about it; and [4] don't just observe in the field, do field experiments. This is what we have done and we believe that this approach is appropriate for EEM applications.

The $500,000.00 Fish. B. Zajdlik[1], G. Gilron[2], P. Riebel[3] and G. Atkinson[4]. [1]Zajdlik Associates, Rockwood, ON; [2]ESG International, Guelph, ON; [3]Riebel & Associates, Baie d'Urfè, QC; [4]Atkinson Statistical, Calgary, AB.

Compliance decisions regarding effluent toxicity in Ontario are made using a Rainbow Trout and/or *Daphnia magna* pass/fail test following standard methods. Federally, a failure of the Rainbow Trout pass/fail test invokes estimation of an effluent Rainbow Trout LC_{50}. The Rainbow Trout and *D. magna* pass/fail tests are good indicators of toxicity but the paradigm for testing compliance is contentious in several respects. One of the more fundamental issues is the significance of the death of one additional fish (or possibly a daphnid) in triggering a non-compliance decision. The death of this organism may result in a maximum fine of $500,000.00 (that we are aware of) and adverse public relations for the discharger. Fines are rarely based on the results of a single pass/fail test. However, even in cases where fines are levied due to multiple failures, there is contention in cases where non-compliance is due to the death of the 6th fish (or 16th daphnid). We feel that is in the best interest of stakeholders to reduce this contention, by understanding the false positive and negative rates of the pass/fail tests in the context of the current compliance paradigm. We also discuss changes to the compliance paradigm that reduce the false positive and negative rates. The primary benefit of an improved pass/fail paradigm is increased confidence in the compliance decision among stakeholders.

Identification of Sublethal Toxicants in a BC Coastal Pulp and Paper Mill Effluent. C.V.

Eickhoff[1], J. Pickard[1], K. Kinnee, L.U. Young[2], D.A. Birkholz[3] and D. Kilback[4]. [1]BC Research Inc., Vancouver, BC; [2]Hatfield Consultants Ltd., West Vancouver, BC; [3]EnviroTest Laboratories, Edmonton, AB; [4]Pacifica Papers, Powell River, BC.

A toxicity identification evaluation (TIE) was performed on effluent samples obtained from a pulp and paper mill on the coast of British Columbia. The TIE was conducted to identify the components of the effluent which had caused sublethal toxicity in previous toxicity bioassays involving echinoderm species and *Champia parvula*. The marine fertilization test was adapted to evaluate the toxicity of effluent samples treated using TIE techniques. Fractions were isolated following Ph adjustment and solid phase extraction which caused a significant inhibitory effect on sea urchin (*Strongylocentrotus pupuratus*) egg fertilization. These fractions were further analysed by GC, GC/MSD, LC/MS and Time of Flight (MALDI-TOF) analysis. The main components of the toxic fractions were characterized by mass spectrometric analysis as high molecular weight, non-volatile molecules between 1,000-2,800 Da with irregular fragmentation patterns. It is suspected that these molecules may be amine polymers which were introduced into the pulp and paper process as flocculant products.

Summary of Sublethal Toxicity Test Results from the Second Cycle of the Pulp and Paper Environmental Effects Monitoring Program. R.P. Scroggins[1] and J.A. Miller[2]. [1]Environment Canada, Method Development and Application Section, Ottawa, ON; [2]Miller Environmental Sciences Inc., Stroud, ON.

As a component of the *Pulp and Paper Effluent Regulations*, industry is required to conduct an Environmental Effects Monitoring (EEM) program at mills across Canada to assess whether or not the national effluent regulation was protective of fish populations, fish habitat and the fisheries resource. One of the monitoring requirements for the first cycle (1992-1996) and second cycle (Summer 1997 to Winter 2000) of the EEM program is the measurement of sublethal toxicity in samples of a mill's final treated effluent. During the second cycle, a battery of three toxicity tests were used to assess potential sublethal effects of mill effluent on: [1] early-life-stage development of fish; [2] reproduction of an invertebrate; and [3] growth of an aquatic plant on a twice yearly basis (i.e., summer and winter periods). Throughout Cycle 2, Environment Canada Regional staff examined the quality of the sublethal toxicity results to ensure that method QA/QC requirements had been met and that the test results were valid. Following the QA/QC check, the data were submitted to the National EEM Office for compilation of the results. The Method Development and Applications Section has taken on the task of assessing the performance of the battery of tests and identifying trends in the Cycle 2 sublethal toxicity data set. A summary of freshwater and marine sublethal test data from all Canadian pulp and paper effluent discharges will be presented with discussion focussed on test sensitivity trends, comparability of results between freshwater and marine tests, temporal changes in effluent quality and any apparent national trends between species-specific test results and effluent treatment system type.

Pulp and Paper Environmental Effects Monitoring (EEM): Program Changes Since Cycle 2. E. Porter and I.K. Ellis. Environment Canada, National EEM Office, Hull, QC.

Environment Canada is proposing a series of amendments to the *Pulp and Paper Effluent Regulations* (PPER) under the *Fisheries Act* that will result in some changes to the EEM program. While a number of these amendments stem from advances in EEM science, others are the result of the recent development of a similar EEM program for the metal mining industry. This presentation will focus on the technical revisions to the Pulp and Paper EEM requirements and their influence on the EEM Program since Cycle 2. Technical changes to the pulp and paper EEM requirements include a modified EEM objective and definition of "effect"; new options to fulfill the fish survey requirement in addition to the use of traditional large bodied adult fish (e.g., use of forage and immature fish, non-

lethal surveys); a new requirement to conduct an alternative when the traditional approaches for the fish or benthic invertebrate surveys can not be achieved, and a clearer explanation of the monitoring requirements once the presence or absence of an effect has been determined.

A "Local" Regional-Reference Design to Assess Benthic Macroinvertebrates of the Exploits River, NF. B.W. Kilgour[1], C.B. Portt[2], G. Coker[2] and I. Cloutier[3]. [1]Water Systems Analysts Inc., Guelph, ON; [2]C. Portt and Associates, Guelph, ON; [3]Abitibi-Consolidated Inc., Montréal, QC.

The use of local reference sites for assessing benthic communities downstream of point source discharges can lead to erroneous conclusions of impact when upstream and downstream habitats naturally differ. Because of this conundrum, the regional-reference approach is being proposed as an alternative. With regional-reference designs, biological responses at "pristine" locations are correlated to physical/chemical habitat features. Using the derived model, biological responses downstream of the point-source discharge are predicted. Since conditions upstream of a point-source are not always pristine, the use of regional-reference designs can also lead to erroneous conclusions of impact. In an EEM study for the Abitibi-Consolidated Inc. mill at Grand Falls, Newfoundland, a compromise design was used. Because of natural differences in habitat between upstream and downstream locations, natural differences in the benthic community were predicted *a priori*. Fifteen upstream sampling stations, located close enough to the mill to reflect the local background water quality, were selected to vary in water depth and velocity, so as to enclose the variability observed in the exposure area. A model developed using the data from the upstream stations was used to predict expected indices of benthic community composition for the exposure stations. The benthic community in the exposure area was more abundant and diverse than predicted, and had several taxa requiring high water quality. The observed response was consistent with moderate enrichment.

Use of the Beak Artificial Sand Substrate ("BASS") Sampler to Eliminate the Confounding Effects of Historic Deposition. B. Fraser[1], D. Farara[1], M. Lacey[2] and S. Peddle[3]. [1]Beak International Inc., Brampton, ON; [2]Corner Brook Pulp and Paper Inc., Corner Brook, NF; [3]Humber Arm ACAP, Corner Brook, NF.

In order to determine whether current effluent quality from a mill affects benthic community structure *in situ*, the effects of historic sediment degradation must be separated from potential current impacts. In many cases, decades of accumulations of industrial and/or municipal waste render conventional benthic sampling devices (e.g., grabs, dredges) inappropriate. For the ICS at Corner Brook Pulp and Paper, benthos were collected with BASS samplers, a device designed for collecting benthos in depositional areas where conventional methods are unable to distinguish between the impacts of current versus historical discharges. Unlike previous surveys, including Cycle 1, the benthic community in the Cycle 2 exposure areas was not dominated exclusively by pollution tolerant taxa and the community generally resembled reference area benthos. The differences observed between Cycles 1 and 2 can be explained by the sampling method used. In Cycle 1, benthos were collected with grabs from a bottom environment that was known to be historically severely impacted. The Cycle 1 results therefore, likely were attributable to poor sediment quality as the result of historical discharges. Conversely in Cycle 2, use of the BASS samplers eliminated the effects of historically poor sediment quality and only the potential effects of current water quality conditions were evaluated.

Bioremediation

Session Chair: K. Lee

Application of Chemical Analysis and Biotests to Monitor Treatment Efficacy and Habitat Recovery of an Experimentally Oiled Freshwater Wetland. K. Lee[1], A.D. Venosa[2], J.R. Haines[2] and M.T. Suidan[3]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [2]National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH; [3]Department of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, OH.

Freshwater wetland environments provide nursery support for coastal fisheries, a habitat for wildlife and endangered species, and a protective mechanism for shoreline erosion. As information on the potential impact of oil spills on north-temperate wetlands are limited, a collaborative experiment was initiated to determine the natural recovery rate and to assess the feasibility of bioremediation and phytoremediation as remediation strategies. A controlled oil spill experiment was conducted in the summer of 1999 at a wetland site situated along the St. Lawrence River, at St. Croix, Quebec, Canada. The study area consisted of 20 experimental plots (5 m x 4 m) dominated by *Scirpus pungens*. Experimental treatments included a no oil control and 4 oiled treatments. The oiled treatments included a natural attenuation (no treatment) control, nutrient amendment with granular ammonium nitrate and super triple phosphate, a similar treatment with plants continuously cut back to suppress plant growth and a treatment consisting of amendments of sodium nitrate rather than ammonium nitrate. A weathered light crude oil was applied in early summer at the rate of 12 L per plot. To ensure that nutrient limitation was not a factor suppressing treatment effects, nutrients were reapplied when interstitial nitrogen concentrations fell below 5-10 mg/L. To monitor the extent of habitat recovery, time-series sampling of sediments for chemical (GC/MS analysis with analytes normalized to hopane) and biological/toxicological analysis (plant survival/growth and microscale bioassays) was initiated one week after oiling, concurrent with the first nutrient application event. Samples were recovered over a 21 week period that effectively covered the natural growth season of the plants.

Remediation of Oiled Beach Sediments as Indicated by Effects on Fish. S. Zambon[1], I. Ibrahim[1], T. Chan[1], Y. Kiparissis[1], K. Lee[2], A.D. Venosa[3] and P.V. Hodson[1]. [1]Queen's University, Kingston, ON; [2]Department of Fisheries and Oceans, Mont-Joli, QC; [3]USEPA, Cincinnati, OH.

To investigate the effect of nutrient enrichment on the disappearance of oil from a freshwater tidal beach, weathered medium MESA oil was applied June 10, 1999 at 0.6 L/m^2 to experimental plots in a zone of *Scirpus americanus* (grass). Plots were unoiled, oiled with no added nutrients, or oiled plus repeated applications of super-triple phosphate and ammonium nitrate or sodium nitrate. Oil was raked in to simulate wave action, and plants in one fertilized plot were cut repeatedly to assess the influence of plant growth. Sediments were sampled 1, 50, 141, and 344 d post-oiling and tested in the lab, diluting with clean sediment to create exposure gradients. Bioavailability of PAH was assessed by 4 d exposures of trout to 320 g of sediment /10 L of water, after which liver MFO activity, an indicator of PAH uptake, was assayed. Oil alone, oil mixed with sediments in the lab, and oiled sediments from treated plots all increased activity relative to controls, indicating the bioavailability of PAH. Induction did not vary with treatments, declined slightly from June to July, and remained unchanged in October, suggesting that nutrient addition or warm season weathering caused only a slight loss of PAH from oiled plots. Preliminary results indicate that oiled sediments are lethal to medaka early life stages unless diluted 50%.

Response of an Intertidal Sediment Microbial Community to Nutrients Following a Controlled Oil Spill. C.W. Greer[1], N. Fortin[1], R. Roy[1], D. Beaumier[1], C. Masson[1], D. Ouellette[1], G. Wisse[1], A. Mihoc[1], S. Labelle[1], L.G. Whyte[1] and K. Lee[2]. [1]National Research Council Canada, Montreal, QC; [2]Department of Fisheries and Oceans, Mont-Joli, QC.

The population density and activity of a microbial community associated with the sediment and rhizosphere of an intertidal freshwater beach populated by *Scirpus americanus*, was monitored before and following the application of weathered medium crude oil and fertilizers to experimental plots. The viable bacterial population density showed a slight increase during the first 4 weeks following oil and fertilizer application, which was consistent with fertilizer application, in contrast to population densities in untreated areas, which remained relatively unchanged throughout the monitoring period. The microbial populations demonstrated a rapid and sustained increase in naphthalene mineralization activity in the fertilized and oiled plots: the activity was somewhat lower in unfertilized/oiled and fertilized only plots. Hexadecane mineralization activity increased in response to fertilizer application, especially ammonium nitrate, in comparison to sodium nitrate: activity in the unfertilized/oiled plots and unoiled areas remained relatively low. Field and laboratory evaluation of nitrogen metabolism indicated significant denitrification activity in the sediments following fertilizer application, which was not adversely affected by oiling. The results demonstrated that the application of fertilizers stimulated the activities of indigenous hydrocarbon degrading and denitrifying bacteria, and the presence of oil did not have a detrimental effect on these activities.

The Response of *Spartina alterniflora* to No. 2 Fuel Oil Exposure. M. Engel[1], K. Lee[2], T.L. Huang[3], M.T. Suidan[3] and A.D. Venosa[4]. [1]Department of Botany, University of Toronto, Toronto, ON; [2]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [3]Department of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, OH; [4]National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Coastal salt marshes are delicate habitats threatened by anthropogenic sources of pollution such as those generated by the fuel production and transportation industries. A greenhouse study using healthy *Spartina alterniflora* transplants in closed containers was conducted in order to examine plant growth after separately exposing aerial and below-ground plant tissue to No. 2 fuel oil. Nine concentrations of oil, ranging from 1.78-114 g/L, were pre-mixed into the growing medium (a mixture of homogenized salt marsh sediment and commercial potting soil) for below-ground plant tissue exposure. Oil was directly applied to 1/3, 2/3 and 3/3 of the aerial photosynthetic tissue to achieve above-ground plant tissue exposure. Results indicated no significant difference in canopy height or plant stem density relative to controls at sediment oil concentrations up to 14.3 g/L. Transplants survived up to 30 d in sediments containing 42.9 g/L oil. Immediate mortality of transplants was observed for sediment oil concentrations above this level. In aerially exposed plants, regeneration occurred through tiller growth, but rates reached no more than 50% of controls in all cases. The influence of surviving plants on biodegradation rates of residual oil within the sediment was determined by gas chromatography/mass spectroscopy (GC/MS) analysis of specific compounds relative to that of 5-α-cholestane which was added to the test oil before its application.

The Mystery Snail, *Viviparus georgianus*, as Biomonitors of Oil-spill and Bioremediation Strategies in Fresh-water Habitats. L.E.J. Lee[1], J. Stassen[1] and K. Lee[2]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [2]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC.

The prosobranch gastropod, *Viviparus georgianus*, are ideal fresh-water "biomonitors" because they are short lived, are viviparous, thus their young remain within the area, and grow rapidly during summer months feeding on algae, diatoms and sediment debris. *V. georgianus* was used as a biomonitor in a controlled oil spill experiment at a wetland site along the St. Lawrence River (Ste. Croix, QC) to assess the impact of crude oil, rates of natural recovery and the efficacy of bioremediation treatments to enhance bacterial degradation of residual oil in the sediments. Snails were placed at various time intervals in special enclosures deployed within five treatments and control

background plots (n=50/treatment/exposure time). Treatments consisted of: [A] oiled control; [B] as A + ammonium nitrate + triple superphosphate + culling of plants; [C] as B but plants left intact; [D] as C but sodium nitrate instead of ammonium nitrate; and [E] as C with no oil treatment. Samples were collected and examined at monthly intervals. Survival was high within the control enclosures (F) with mortality only at 3% after one month, however, mortality among treatments varied from 23% to as high as 82%. Growth and reproductive success were evaluated by gross morphology and histology.

Comparative Bioanalytical Assessment of Several Solid Phase Toxicity Tests Conducted on Oil-Contaminated Freshwater Sediments. C. Blaise[1], N. Chèvre[1], K. Lee[2], J. Lappalainen[3], G. Persoone[4], B. Belgis[4], K.G. Doe[5] and J.R. Haines[6]. [1]Environment Canada, Centre Saint-Laurent, Montreal, QC; [2]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [3]Department of Biotechnology, University of Turku, Turku, Finland; [4]University of Ghent, 22 Plateaustraat, Ghent, Belgium; [5]Environment Canada, Environmental Science Center, Moncton, NB; [6]National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

In support to a bioremediation project undertaken on crude oil-contaminated freshwater (St-Lawrence River) shoreline sediments (Ste-Croix River, Province of Québec, Canada), we evaluated, with the collaboration of partner laboratories, the performance of recent/novel microbiotests to detect the toxicity of samples (whole sediment) collected from the study site. Small-scale toxicity tests assessed included: [1] the Microtox® solid phase assay (Azur Environmental, U.S.A.); [2] the Biotox® solid phase test (Bio-Nobile Oy, Finland); [3] the Algal solid phase assay (Blaise and Ménard, 1998); and [4] the Ostracod solid phase assay (University of Ghent, Belgium). Toxicity data generated with these micro-scale assays were also compared with those obtained with the conventional (reference) endobenthic amphipod bioassay conducted with *Hyalella azteca*. Findings of this on-going comparative study will be presented during the Workshop.

Effects of Various Remediation Treatments on the Toxicity of Oiled Sediments to the Freshwater Amphipod, *Hyalella azteca*. K.G. Doe[1], P.M. Jackman[1] and K. Lee[2]. [1] Environment Canada, Moncton, NB; [2] Department of Fisheries and Oceans, Mont-Joli, QC.

An experiment was conducted at a freshwater marsh in Ste Croix, Quebec, to investigate the effect of various bioremediation treatments on toxicity of oiled sediments to the freshwater amphipod, *Hyalella azteca*. Weathered medium MESA crude oil was applied on June 10, 1999 at 0.6 L/m^2 to replicated treatments in the marsh. There were four replicates of each of five treatments: [1] unoiled; [2] oiled with no added nutrients; oiled plus super-triple phosphate and ammonium nitrate; [3] the same treatment with plants cut repeatedly to assess the influence of plant growth; and [4] oiled plus super-triple phosphate and sodium nitrate. Sediments were sampled at various times up to one year after the initiation of the experiment and tested with the freshwater amphipod, *H. azteca* to determine effects on survival and growth after 14 d exposures to the whole sediment. Unoiled plots were not toxic, but both survival and growth were severely depressed in all oiled treatments at day 1 and day 8. Survival and growth recovered in the sodium nitrate treatment by day 147, whereas treatments using ammonium nitrate and natural attenuation did not recover as fully. Possible reasons for these different recovery rates will be discussed.

Toxicological Risk Assessment of a Simulated Oil Spill with SPMD-TOX. B.T. Johnson[1], J.D. Petty[1], J.N. Huckins, K. Lee[2], J. Gauthier[2] and A.D. Venosa[3]. [1]USGS, Columbia, MO; [2]Department of Fisheries and Oceans, Mont-Joli, QC; [3]USEPA, Cincinnati, OH.

To evaluate the impact of oil on an intratidal wetland a simulated spill was initiated June 10, 1999, along the St. Lawrence River at Ste. Croix, QC. The randomized block experimental design consisted of four replicates of five different conditions: one site was non-oil treated and the other four sites were oiled and each given a different treatment: natural attenuation (no nutrients added), ammonium nitrate + tri-super phosphate (commercial fertilizer) + intact plants, ammonium nitrate + tri-super phosphate + cut plants, and sodium nitrate + tri-super phosphate + intact plants. Weathered Mesa light crude (12 L) was sprayed and manually raked into the top 2-3 cm of each treatment block (4 x 5 m). Changes in toxicity of the experimental sites during weathering and degradation were monitored with SPMD-TOX. SPMD, a semipermeable membrane device collected and concentrated the oil products and Microtox® Basic Test, a microscale toxicity test, determined the changes in acute toxicity (TOX). SPMD units (12.5 cm) enclosed in protective cases were deployed at the water:sediment interface at 0, 1, 3, 5, 7, 11, 15, and 20 weeks and retrieved after one-week periods. SPMD concentrated samples were diluted with organic solvents for toxicological analysis by Microtox. Changes in acute toxicity over time of the oil spill area were detected with SPMD-TOX.

Emerging Issues and Application of New Technologies

Session Co-chairs: R. Buchanan and S. McKinley

Using Caged Bivalves to Monitor Canadian Effluents. M.H. Salazar and S.M. Salazar, Applied Biomonitoring, Kirkland, WA.

Caged bivalves have been proposed for monitoring industrial and municipal effluents in Canada, and Environment Canada is considering this approach as an alternative to the adult fish survey. Caged bivalves have a long history of use throughout the world, including Canada. Over the past three years we conducted caged bivalve studies at the Port Alice pulp mill on Vancouver Island and near municipal effluents in Winnipeg and Montreal. Others have used our methods in both freshwater and marine environments in Canada for monitoring pulp and paper mill effluents. Scientists in Finland have been using caged bivalves to monitor their effluents since the mid-1980s.

The goals of Environmental Effects Monitoring (EEM) are similar to most assessments and similar across pulp and paper, mining, and offshore oil and gas platforms: [1] characterize exposure; [2] characterize effects; [3] quantify these relationships over space and time; and [4] evaluate whether conditions are getting better, worse, or staying the same. The approaches are also similar, characterize and understand ecological processes occurring under site-specific conditions and develop a monitoring program capable of predicting adverse environmental effects.

The problems are also similar; there is too much uncertainty in traditional methods. Laboratory bioassays do not adequately assess the potentially ameliorating or enhancing effects of receiving waters and test conditions may not be at steady state. Benthic community structure is generally at steady state but the exposure period and associated effects may be difficult to establish. Exposure in adult fish surveys is largely unknown and unquantified. There is a need to quantify site-specific exposure and effects under steady-state conditions.

Field studies with caged bivalves combine the of experimental control characteristic of laboratory bioassays with the environmental realism characteristic of traditional field monitoring: [1] measurements are site-specific; [2] exposure is clearly defined because animals are deployed and removed at specific times, they are deployed at specified positions and cannot move because they are caged; [3] the fixed deployment position and period facilitates hypothesis testing because there are fewer uncertainties than traditional field monitoring; and [4] test species are always available and the same species can be used in different environments. In addition to using caged bivalve studies

for field validation of laboratory bioassays, they can be used to establish testable null hypotheses under real-world conditions. Chemicals in mussel tissues provide a direct method of mapping and monitoring effluents and defining mixing zones because bioaccumulation integrates biologically available chemicals over time. In contrast, it may be difficult to accurately map effluent plumes or define mixing zones with even thousands of water samples because of the ephemeral nature of most water masses — the concentrations may be too low for detection by analytical techniques or peak concentrations may not coincide with collection times. Analysis of water samples does not allow for quantification of bioavailable chemicals or integrated estimates of chemical exposure. Without a realistic characterization of exposure, the characterization of effects is less useful than it could be.

Port Alice Mussel Study

Applied Biomonitoring was contracted by Environment Canada to conduct a caged bivalve pilot study at a pulp and paper mill on Vancouver Island in British Columbia. Fig. 1 shows the deployment configuration for the Port Alice study. Paired stations were positioned at approximately 0.3, 3 and 10 km from the effluent diffuser. At each of these locations, mussel cages were placed approximately 100 m apart. Each cage contained 90 mussels; cages were placed at depths of 2, 4 and 6 m below the surface. Previous monitoring by the mill had shown that this is where the effluent plume was expected. Mussel tissues were analyzed for several compounds speculated to be tracers of mill effluent.

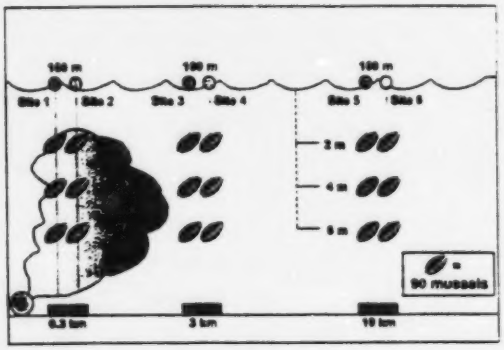

Figure 1. Port Alice deployment.

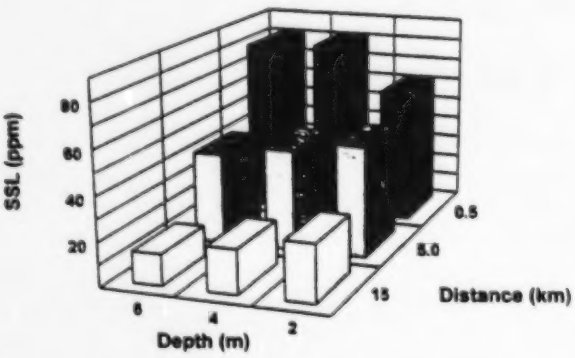

Figure 2. Spent sulphite vs distance & depth.

Spent sulphite liquor varied with depth and distance from the effluent diffuser (Fig. 2). The highest concentrations were found closest to the diffuser and at the 6 m depth. The lowest concentrations were found furthest from the mill and at the 6 m depth. These differences were attributable to the SSL rising to the surface as the plume moved away from the bottom diffuser. Mussel growth rates increased with distance from the diffuser and decreased with depth (Fig. 3). These data suggest that some component in mill effluent reduced mussel growth. It seems likely that decreasing temperature decrease mussel growth with depth. A statistically significant correlation between campesterol in mussel tissues and mussel growth rate was found (Fig. 4). This suggests that campesterol may be a tracer of mill effluent. This correlation does not demonstrate a cause-and-effect relationship but at the very least provides a working null hypothesis for future monitoring.

Table 1 shows that different mussel metrics provided somewhat different groupings of mussel growth at paired sites. All five metrics shown here demonstrate that mussel health increased with distance from the mill. Whole animal weight and length growth rates suggested that the furthest sites were

similar while the end-of-test tissue weights and percent lipids in mussel tissues suggest that the closest stations were most similar.

Table 1. Port Alice mussels - Summary of results of pooled stations.

	Pooled stations		
	1&2	3&4	4&5
Weight growth rate (mg/week)	218	236	248
Length growth rate (mm/week)	1.22	1.28	1.32
EOT tissue weight (g-wet)	0.68	0.69	0.76
EOT percent lipid (%)	1.00	1.14	1.48
EOT percent water (%)	79.5	78.4	77.5

—— = statistically similar group

A gradient of decreasing mussel growth with proximity to the diffuser was correlated with a number of physical-chemical factors such as spent sulphite liquor, dissolved oxygen, and temperature as well as campesterol (a plant sterol) in mussel tissues. Since a number of these factors co-varied, more work will be necessary to confirm meaningful relationships and establish the causative factors for reduced mussel growth near the diffuser.

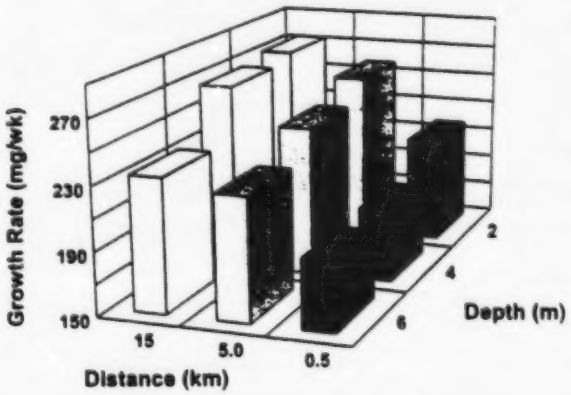

Figure 3. Mussel growth rate vs distance & depth.

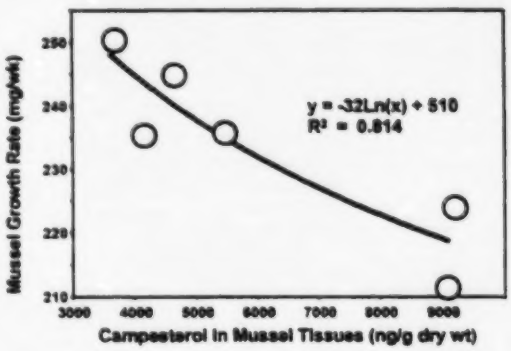

Figure 4. Mussel growth vs campesterol.

Winnipeg Mussel Study

The Winnipeg caged mussel study was conducted for the City of Winnipeg by TetrES Consultants in cooperation with Gordon Craig & Associates and Applied Biomonitoring. Gordon Craig was responsible for the laboratory testing and Appiied Biomonitoring was responsible for the field testing with caged mussels. Modeling work by TetrES provided a prediction of plume dispersion with distance and facilitated the placement of cages at strategic locations upstream and downstream on the Red River (Fig. 5).

Fig. 6 provides a more detailed schematic and shows that the cages were placed at regular intervals from the diffuser. Each cage contained 39 floater mussels (*Pyganodon grandis*) and 100 fingernail clams (*Sphaerium similie*). Fingernail clams were used since the US EPA ambient water quality criterion document showed that fingernail clams were the second most sensitive species with respect

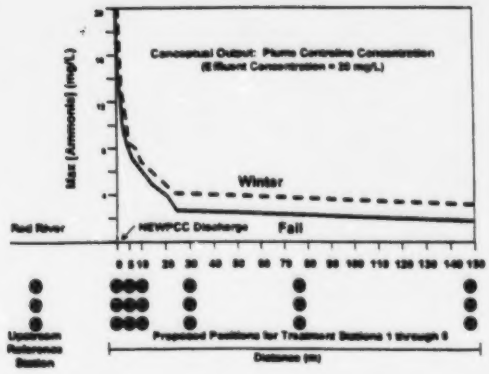

Figure 5. Winnipeg mussel stations.

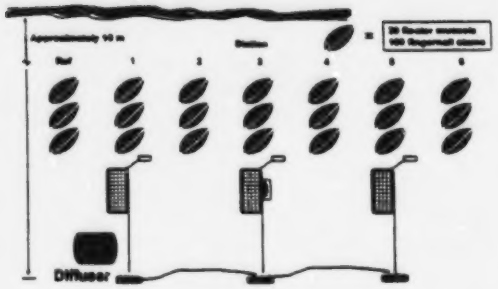

Figure 6. Schematic of mussel deployment.

to chronic toxicity. However, a careful scrutiny of the data suggested that some of this apparent sensitivity to ammonia may have been attributed to laboratory-induced stress. Since one objective of the Winnipeg study was to establish site-specific ammonia criteria, it became important to re-evaluate provincial and national criteria as well as US EPA criteria for ammonia using only species found in the Red River and its tributaries. Subsequent laboratory and field studies demonstrated that existing criteria were far too low and that laboratory tests with bivalves in particular over-estimated potential toxicity.

Montreal Mussel Study

Previous studies of the City of Montreal municipal effluent by Environment Canada scientists have shown a number of potential adverse biological effects, including MFO induction in fish. Christian Blaise and Francois Gagne of Environment Canada pioneered the use of freshwater bivalve biomarkers to provide further insight into these potential adverse effects. While the data are still being evaluated, preliminary information will be presented here.

Table 2. Montreal mussels - Growth rates

	1999		2000	
	Upstream	Down	Upstream	Down
% Survival	100	100	92.5	92.5
% ¯ Weight	1.35*	2.69	1.96	1.57
% Δ Length	0.56*	-0.29	-0.07	-0.08
EOT tissue weight (g-wet)	5.85*	6.40	4.95*	5.40
EOT shell weight (g-wet)	15.28	14.93	15.92	15.98
WAWW growth rate (mg/week)	43*	88	45	35

* = Statistically significant difference ($P<0.05$)

Biomarkers measured as part of the 1999 and 2000 studies included estrogenicity, coprostanol, phagocytosis, total bacteria, gonadal, LPO, DNA damage, metallothionein in the hepato-pancreas and in the gill, MFO induction (EROD), and vitellins. It is hoped that the work on vitellins by Francois Gagne will allow us to use the caged bivalve methodology to establish more links with the adult fish survey. One criticism of the caged bivalve surveys with respect to EEM has been that it has not generally included a reproductive endpoint. Inclusion of vitellins may help satisfy that need.

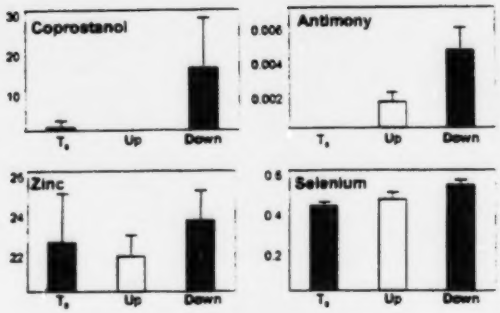

Figure 7. Montreal Mussels - Tissue chemistry higher downstream.

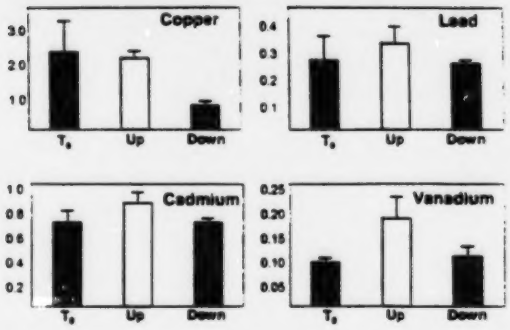

Figure 8. Montreal Mussels - Tissue chemistry higher upstream.

Growth results (Table 2) show significant differences between upstream and downstream sites and differences between 1999 and 2000. Percent change in whole- animal wet-weight and weight growth rates were significantly higher downstream in 1999; approximately two times higher. Only end-of-test tissue weights were significantly higher downstream in 2000. Whole-animal weight growth rates were not significantly different, but they were higher upstream in 2000. These data suggest that there may have been temporal differences in chemical exposure and associated biological effects. It is hoped that pairing growth and biomarker measurements will help understand these seasonal differences.

As part of characterizing chemical exposure, heavy metals and organic chemicals in mussel tissues were measured in 1999 and 2000 (2000 results are not yet available). In 1999, coprostanol, Sb, Zn and Se were significantly higher downstream (Fig. 7) while Cu, Pb, Cd and V were significantly higher upstream (Fig. 8).

Threatened and Endangered Species

While the Port Alice caged mussel study was conducted in marine waters, both the Winnipeg and Montreal studies were conducted in freshwater. This adds other complicating factors with respect to EEM. Many Canadian freshwater mussel species are threatened and endangered. This makes it even more important to monitor these bivalves as part of EEM. In the US, freshwater mussels have been shown to be the most imperiled animal group; not fish, not birds, mammals, or insects. Approximately 70% of the species are threatened or endangered and 20% of the species are presumed extinct. It has been predicted that another 10% may become extinct in this century. Freshwater mussels are particularly sensitive to anthropogenic stresses because of their reliance on a fish host for reproduction. Their numbers have declined due to dredging, filling, diking and other activities of municipal populations that have caused a loss of habitat for either the mussels or their fish hosts. The most successful species are those who have multiple fish hosts and the least successful those who only have one. Like the canary in a coal mine, freshwater mussels are indicators of environmental quality and their declining numbers suggest that there is a significant environmental problem associated with loss of habitat or the introduction of chemical stressors or both.

Summary

The Port Alice study showed that caged bivalves separated by only two meters vertical distance could identify the fine structure of exposure and associated biological effects. The Winnipeg study showed that caged bivalves at field sites survived considerably better than those in the laboratory and that previous laboratory studies have probably over-estimated the effects of ammonia under real-world

conditions. The Montreal study showed that caged bivalves were a good indicator of exposure, and Environment Canada scientists demonstrated a relationship between mussel tissue chemistry and a suite of biomarkers. Collectively, these studies have validated the operational utility of caged bivalve monitoring. A monitoring framework has been developed using tissue chemistry to establish links with measurement endpoints in other species like fish. Collectively, these studies show that caged bivalve monitoring can be a useful tool as part of EEM for pulp and paper effluents and municipal effluents. Furthermore, this methodology can provide important information not provided by traditional methods. It also provides the following: [1] a method for validating the results of laboratory studies; [2] developing null hypotheses for additional testing; and [3] an approach for developing site-specific ambient water quality criteria.

Effects of a Municipal Wastewater Discharge on Mussels. M.D. Paine[9] and C. Larose[2]. [1]Paine, Ledge and Associates (PLA), North Vancouver, BC; [2]Environmental Services Group, Capital Region District (CRD), Victoria, BC.

Horse mussels (*Modiolus modiolus*) were collected in September, 1998, from 15 stations within 800 m of a marine municipal wastewater outfall, and from a reference station. Concentrations of Pb and Cu in mussel tissue decreased with increasing distance from the outfall (=expected gradient). Concentrations of other metals either showed no relationship with distance (=no gradient), or increased with increasing distance from the outfall (=reverse gradient). Similar results were observed in surveys conducted in 1995 and in 1992. Some, but not all, reverse gradients could be attributed to growth dilution. Growth dilution occurs when tissue mass increases at a faster rate than metal mass; concentrations are metal mass/tissue mass. Mussels near the outfall grew faster than mussels at more remote stations, presumably because they were feeding on organic matter from the discharge. Reverse gradients might also occur if metals were competing for binding sites. Finally, no gradients were expected for several metals that do not occur at elevated or above-background concentrations in the effluent. Since there was no evidence of adverse biological effects of the discharge on the mussels, the major concern at this site would be indirect effects of Cu and Pb in mussel tissue on predators. Wild mussel surveys are a useful monitoring tool at this and other sites, where fine sediments and soft-bottom benthic invertebrate communities are rare.

Cytochrome P4501A Responses in Two Sub-Tropical Fish of Bangladesh, Riverine Catfish (*Rita rita*) and Marine Mudfish (*Apocryptes bato*). S.A.M. Al-Arabi[1,2] and A. Goksøyr[3]. [1]Department of Fisheries and Marine Biology, University of Bergen, Bergen Norway; [2]Bangladesh Fisheries Research Institute, Brackishwater Station, Khulna, Bangladesh; [3]Department of Molecular Biology, University of Bergen, Bergen, Norway.

The present research study focuses on the establishment of methods for biomarker studies in freshwater and marine fish species as a basis for monitoring the extent of contamination of fisheries resources in Bangladesh. Riverine catfish (*Rita rita*) and marine mudfish (*Apocryptes bato*) were given a single intraperitoneal injection with two selected inducing compounds; ß-napthoflavone (BNF, 50 mg/kg), Clophen A50 (Clo A50, 20 mg/kg) and the heavy metal compound cadmium chloride ($CdCl_2$, 1 mg/kg). Effects on cytochrome P4501A (CYP1A) were determined in post mitochondrial supernatants (PMS) of liver at days 3 and 10 after treatment. EROD (7-ethoxyresorufin O-deethylase) activity and CYP1A protein level by indirect non-competitive enzyme-linked immunosorbent assay (ELISA) and by western blotting using a monoclonal antibody against fish CYP1A, FA-1, were measured. BNF and Clo A50 resulted in good induction (up to 9.5-fold and up to 5-fold) of CYP1A protein, while $CdCl_2$ showed significant (up to 16.5%) inhibition in these species. The present study examined phase-I cytochrome P450 monooxygenase activity and response, in these two sub-tropical fish species for the first time.

Measurement of Fatigue and Recovery Using EMG Telemetry in Rainbow Trout (*Onchorhynchus mykiss*) Exposed to Acute Chlorine Stress. K.J. Hunter and R.S. McKinley. Department of Biology, University of Waterloo, Waterloo, ON.

Environmental stress can often elicit the escape response, which can induce fatigue upon the animal. The time to fatigue is affected by the fish's level of stress before the environmental stressor affected the fish. The objective of this study was to examine the effect of chloramine on time to fatigue rainbow trout using EMG telemetry. Time to fatigue can be monitored in the lab by following levels of blood lactate, glucose, and pH. The levels of these blood parameters and chloramine levels were correlated to conduction velocity, measured with hardwired EMG, and to the output of the current EMG transmitters, as would be necessary for field experiments. The fish were exposed to 0, 0.5 and 1 mg/L chloramine and subjected to forced swimming. Results suggest that increased chloramine exposure causes decreases in swim performance as indicated by lower EMG based conduction velocities. Chloramine exposure failed to yield significant correlation to the direct output of the current EMG transmitter. Therefore EMG telemetry can, with some modification to its present form, be utilized to detect acute chloramine exposure.

Assessing the Impact of Low Level and Chronic Toxicity on Macroinvertebrate Communities in Rivers. M.H. Colbo. Department of Biology, Memorial University of Newfoundland, St. John's, NF.

The challenge in quantifying the composition and abundance of benthic macroinvertebrates in rivers is still a major obstacle to assessing the ecological impacts of toxic substances on these communities. Extensive and varied sampling by experienced collectors can provide good presence/absence data. However, collecting good quantitative abundance data in streams that reflect the true composition of the benthic community is, for the most part, not achievable due to sampling difficulties and the variable nature of the river habitat. In addition no two streams are the same, making replication difficult, particularly for the long term studies needed to demonstrate low level cumulative effects. It is necessary to recognize these limitations and design monitoring protocols that are perhaps less ambitious but yield results that are easier to interpret. The paper will discuss the potential use of artificial substrate samplers and their limitations in this regard including rock bags. It will also be suggested that using artificial samplers monitoring should shift to the late winter in much of Canada as flow under the ice is stable, thus distribution of the invertebrates are more stable than during the more fluctuating flow in ice free periods. The second value of the end of winter monitoring is that all populations have developed over much of their growth at approximately the same temperature. Therefore the variance between years in abundance due to population dispersal by rapid flow change should be low and developmental parameters should better reflect pollutant stress where temperatures have been relatively constant. To increase this advantage the program should select taxa that undergoes considerable growth during the winter period.

Development and Application of Habitat – Benthic Community Structure Models for Assessing Biological Impairment of Disturbed Sites. L.C. Grapentine. Environment Canada, National Water Research Institute, Burlington, ON.

Assessments of biological conditions in disturbed sites often must be performed without pre-disturbance data. In such cases, direct determination of impairment is not possible because the undisturbed condition specific for each site is not known. Impairment must instead be inferred by comparison to the "expected" undisturbed condition, which is defined by observations from other similar, but undisturbed reference sites. Definition of the undisturbed condition can be improved by the use of predictive, multiple regression models that relate environmental variables to community

structure. Expected community structure for a test site is obtained by first determining the relationship between community structure descriptors and habitat variables for the reference sites, then applying the models to the test site. If the observed community structure significantly deviates from that expected for an undisturbed condition corresponding to the test site, the community is considered impacted. By accounting for variability related to differences in natural environmental conditions among sites, the expected undisturbed condition is more precisely defined, and the power to detect impairment is increased. This approach performed well for assessing benthic community conditions of nearshore contaminated sites in the Great Lakes.

Fish Parasites as Bioindicators of Pollution. R.A. Khan. Department of Biology and Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF.

During the last two decades, both laboratory and field studies have suggested that fish parasites can be useful as bioindicators of pollution following chronic exposure. A number of recent observations provide further evidence that a bottom-dwelling flatfish, the winter flounder (*Pleuronectes americanus*) is a species sensitive to environmental change in habitats degraded by pollutants and responds by exhibiting external and tissue lesions and changes in its parasitic fauna. Studies conducted on this fish sampled near two pulp and paper mills, an oil refinery and a PCB-contaminated naval facility revealed elevated levels of lesions, detoxication enzymes and more external but fewer internal parasites than reference groups. Field trials which involved sampling of flounder at different distances from the outfall of a pulp and paper mill and long term exposure of other groups in the laboratory to varying concentrations of crude oil-contaminated sediment indicated a gradient response of lesions, enzymes and parasites. These results support the view of parasites as an additional bioindicator in multidisciplinary studies on the response of fish to pollutants following chronic exposure.

Diagnosing the Cause of Frequent Fish Kills in Agricultural Areas of Prince Edward Island. J. Mutch[1], W.R. Ernst[2], K.G. Doe[2] and P.M. Jackman[2]. [1]PEI Department of Technology and Environment, Charlottetown, PE; [2]Environment Canada, Atlantic Region, Dartmouth, NS.

Since 1994, there have been fourteen substantial fish kills in agricultural areas of the province of Prince Edward Island and 1999 had the largest number (8). There have been a number of common themes for those kills including: occurrence in mid- to late July; all followed heavy rainfall events; point of origin at potato fields; landwash from fields evident at point of origin; water conditions (temperature and DO) not an apparent factor; healthy fish prior to sudden death with no post mortem signs of disease or trauma. Pesticide sampling has been conducted in association with fish mortality events and at other times. While various pesticides have been detected in water and sediments, because of the ephemeral nature of maximum pesticide residue concentrations, very few residue concentrations have been at or near lethal concentrations, making positive identification of the cause of the kill difficult. In an attempt to develop a post-event diagnostic tool, a number of bioassays were conducted with rainbow trout, to determine the residue concentrations of high risk pesticides in various tissues after exposure to lethal and near lethal concentrations.

Measurement of Immunocompetence in Hemocytes of Bivalve Shellfish with a Miniaturized Assay Based on Phagocytosis Capacity. C. Blaise[1], S. Trottier[1], F. Gagné[1], P.-D. Hansen[2], J. Pellerin[3], M.H. Salazar[4] and S.M. Salazar[4]. [1]Centre Saint-Laurent, Environment Canada, Montreal, QC; [2]Technische Universität Berlin, FB 7 - Institute for Ecological Research and Technology, Department of Ecotoxicology, Berlin, Germany; [3]University of Quebec at Rimouski, Rimouski, QC; [4]Applied Biomonitoring, Kirkland, WA.

Immunocompetent hemocytes in bivalve molluscan shellfish play key roles in maintaining homeostasis which can be disrupted by natural as well as man-made (a)biotic factors. Within a "biomarker battery approach" constructed to investigate the health of mollusks exposed to [a] multiple and diffuse contamination sources (*Mya arenaria* in the Saguenay Fjord); and to [b] a major urban effluent discharging to the Saint-Lawrence river *(Elliptio complanata* cage exposure experiments), we have developed a 96-well microplate immunocompetence biomarker procedure to estimate phagocytosis capacity of hemocytes extracted from posterior adductor muscle hemolymph of such animals. *M. arenaria* collected from a specific site in the Saguenay Fjord apparently subjected to metallic stress displayed immunostimulation associated with marked metallothionein induction in hepato-pancreatic tissue. In contrast, *E. complanata* exposed both in the laboratory for 4 d to a major urban effluent as well as *in situ* for 60 d at a 4 km downstream distance from the outfall demonstrated a significant decrease in immune response. Results suggest that hemocyte phagocytosis performance must be interpreted cautiously because of the potential influence of confounding factors which can modulate its response.

Using the Comet Assay to Assess DNA Damage in Hemocytes and Digestive Gland Cells of Mussels. D. Hamoutene[1], C. Andrews[1], A.D. Rahimtula[2] and J.F. Payne[1]. [1]Department of Fisheries and Oceans, Science Branch, St. John's, NF; [2]Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF.

Maintaining DNA integrity is of paramount importance to all living things. Stresses can result in an increase in DNA damage. The Comet assay has proven to be a rapid tool for collecting data on DNA strand breakage. This method measures the electrophoretic migration of DNA away from the nuclei of cells immobilized in agarose gel. The distance of DNA migration (the comet) is indicative of the number of strand breaks and can be measured using an ocular micrometer or image analysis software. In this study the Comet assay was used to quantify DNA damage in *Mytilus edulis* hemocytes and isolated digestive gland cells. Mussels can often be used as surrogates for assessing potential effects on the health of bivalves, and by considering both hemocytes and digestive gland cells in one individual, a more precise diagnosis can be given on any stress effect. In every test, it is important to obtain "controls" that are reproducible and not altered. Control hemocytes demonstrated small numbers of comets and a variation coefficient between individuals of only 14.04% (n=12). On the other hand, control digestive gland cells showed little DNA damage. Since such damage can be theoretically produced endogenously or during tissue dissociation and processing, it was necessary to try to improve the quality of control cells. No differences in cell numbers were recorded after 1, 2 or 3 h of non-enzymatic tissue dissociation and a mortality rate of 7.77±5.06% was obtained. The effects of protease inhibitors as well as effect of sex on DNA damage is also being considered in trials with mussels exposed to petroleum hydrocarbons and mine tailings. Supported in part by the Program on Energy Research and Development (PERD) and the Toxic Substances Research Initiative (TSRI).

A New Technology for Presenting Whole Industrial Effluent to Fish Cell Lines in Order to Detect Toxicants. K. Schirmer[1,2], V. Dayeh[1] and N.C. Bols[1]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Centre for Environmental Research, Leipzig-Halle, Germany.

Methodology was developed for presenting whole industrial effluent to fish cells in culture without extraction. This was done in order to reduce the labour and use of costly extraction solvents and to ensure that the cells were exposed to all or most potential cytotoxicants present in the effluent sample. This was achieved by adding to the effluent the amounts of salts, galactose and sodium pyruvate, as solids, that were necessary to give concentrations and osmolality of the basal growth medium, Leibovitz's L-15. Non-toxic sample effluents were spiked with known environmental contaminants to determine the cytotoxic response of the fish cells. Chemicals used in this study were

pentachlorophenol, naphthalene, and Triton-X for general cytotoxicity; fluoranthene for photocytotoxicity; and benzo[a]pyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin for aryl hydrocarbon receptor (AhR) active compounds. For cytotoxicity, cell viability was measured with three fluorescent indicator dyes: alamar Blue™, 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM), and neutral red. For AhR active compounds the induction of 7-ethoxyresorufin-O-deethylase (EROD) activity was monitored. Although in some effluents microbes overwhelmed the fish cells, in all other spiked effluent samples the fish cell assays detected general cytotoxicants, photocytotoxicants and AhR active compounds. Filtering the effluents eliminated microbes but still allowed the detection of toxicants. Such bioassays reduce the use of fish in toxicity testing, and in turn, the costs of testing. In addition, the time necessary for cellular responses to develop can be relatively short.

Story of the Toxic Bag. T. Moran[1], C. Ferguson[1], D. Sawchuk[2], D. Hoffman[3] and P. Child[4]. [1]Pollutech Enviroquatics Ltd., Point Edward, ON; [2]Bayer Inc., Sarnia, ON; [3]Shell Canada, Sarnia Manufacturing Centre, Corunna, ON; [4]Investigative Science Inc., Burlington, ON.

Typically effluent compliance toxicity samples are collected into polypropylene pails with polyethylene bag liners. As required by all testing protocols all materials in which samples are collected, transported and stored must be made of nontoxic material. In most cases toxicity testing laboratories use new food grade polyethylene bags to line the polypropylene sample pails. Would it be expected that the food grade polyethylene bags in which samples are collected would be a source of acute and chronic toxicity? A recent incident clearly indicates food grade polyethylene bags could be a source of toxicity of which we all should be aware. The investigation of acute and chronic toxicity detected in several process and cooling water discharges from a refinery complex and a synthetic rubber production facility found the source to be associated with the polyethylene bags. This paper discusses this incident providing a summary of the investigation completed, toxicity identification evaluation methods used and subsequently, the additional procedures implemented to ensure such an incident does not occur again.

Aquatic Toxicology in Canada 1950-2000: Lessons Learned – Future Explored. G.R. Craig[1] and P.G. Wells[2]. [1]G.R. Craig & Associates Inc., Bolton, ON; [2]Environment Canada, Dartmouth, NS.

The half century of major research and technological advances in aquatic toxicology have been highlighted for each decade and regulatory initiatives are identified. Successful monitoring programs and their findings have led to further fundamental changes in environmental protection. The perspective of industry has shifted in response not only to regulatory change but also to societal expectations and market forces. Major events include development of effluent regulations under the *Canada Fisheries Act*, creation of the *Canadian Environmental Protection Act* (CEPA), the *Environmental Assessment Act* (CEA), development of national toxicity test protocols and their role in permits and monitoring programs, new analytical chemistry technology and detection limits, site and sector specific Environmental Effects Monitoring (EEM), effluent toxicant identification/evaluation (TIE), quality assurance/control programs, laboratory certification (CAELA), computer data handling improvements, refinement of risk assessment, the expanding commercial environmental sector and the effect of litigation. All of these events have resulted in changes in attitude and operation within the environmental sectors of government, industry and consulting. Resource management responsibility and technological capability continues to shift from government to industry resulting in the private sector taking ultimate responsibility for environmental protection and preservation. The next century will see increasing global adoption of this philosophy and practice.

Intergovernment Ecotoxicological Testing Group (IGETG): A Review of Accomplishments and

Directions for the Future. P.G. Wells[1] and IGETG Members. [1]Environment Canada, Dartmouth, NS.

The Intergovernmental Aquatic Toxicity Group (IGATG) has been in existence for 25 years and has been instrumental in cultivating and shaping the landscape of laboratory environmental toxicological testing in Canada. The groups' formation was largely in response to the requirements for laboratory toxicity testing under the *Federal Fisheries Act*. The committee that eventually became IGATG was formally established in 1976 as the Technical Sub-Committee of the EPS Toxicity Coordinating Committee. Since this time the Committee has grown to include representation from not only all the Environment Canada Toxicological Laboratories and programmes that use toxicological testing, but also the majority of the Provincial Government laboratories. In 1999 the IGATG group voted on a name change to IGETG (Intergovernmental Ecotoxicological Testing Group) to reflect the changing roles that the laboratories play in sediment and soil toxicological testing. Major achievements from this group will be detailed by the speaker include: [1] Standardization of Toxicological testing methods across Canada; [2] Champion for the use and continued use of Biological test methods for environmental protection and regulation and environmental effects monitoring programmes, and [3] Working with the Canadian Association of Analytical Laboratories (CAEAL) to establish QA/QC, performance evaluation and accreditation programme for toxicological laboratories. In addition to past accomplishments, the presenter will evaluate progress in the field of laboratory toxicology from the IGETG perspective and future directions that IGETG perceives the Canadian toxicological community moving into, such as microscale testing, genomics, endocrine disruptors, and terrestrial testing.

Reporting Results Right! The Facts Concerning Detection Limits and Reliability of Data. D.A. Birkholz[1] and L. Keith[2]. [1]Enviro-Test Laboratories, Edmonton, AB; [2]Waste Policy Institute, Blacksburg, VA.

How results are reported is one of the most controversial areas in environmental analytical chemistry because it affects how data are received, and perhaps more important, how data are perceived and used by the public. Just as important is the sensitive issue of what data are, or are not included in analytical reports. What limits should be used to report a measurement and how analysts and users should handle the resulting data will be the subject of this presentation. The science of modelling and risk assessment has made demands upon laboratories to provide lower detection limits. Laboratories are often asked to provide data which may be beyond their ability to detect and reliably quantify analytes of interest, even with modern and expensive technology. Some examples will be presented and discussed. To complicate matters, detection limits and method detection limits are determined by laboratories themselves, and variances for reported detection limits between laboratories can be large. These variances are not necessarily a function of instrumentation, experience or technique, but rather upon use and/or abuse of protocols prescribed by the US-EPA and the American Chemical Society. Another issue, which has arisen, is effluent permitting. In some instances, freshwater water quality criteria along with effluent dilution factors have been used to set effluent discharge limits. Depending upon the criteria and dilution factors, instances have arisen where limits have been set below the limit of quantitation which makes it difficult to determine whether an industry is out of compliance. The facts concerning the detection and reliability of data will be presented and discussed.

Interpretation and Integration of Biological Data

Session Co-chairs: G. Gilron and H. Sonnenberg

Streamlining Monitoring Requirements at 11 Closed Mine Sites near Elliot Lake, Ontario. P. Stecko, C. Russel and P. Orr. Phoenix.mg Inc., Toronto, ON.

In Elliot Lake, there are 11 closed uranium mines each of which, until recently, had its own receiving environment monitoring program. The affected watershed includes 20 lakes and 30 lake outlets or creeks draining approximately 1,376 km^2. Aggregate annual monitoring costs were approximately $1.5M, focused primarily on water quality with only limited sediment and biological monitoring. The list of parameters measured, the frequency of sampling and sampling locations had not previously been rationalized from an overall watershed perspective. For example, sampling stations were duplicated among mine sites or sampling was undertaken too frequently relative to the timeframe over which environmental changes might be expected to occur. The presentation will outline the watershed-based program that was developed to provide more information at less cost. This allowed the mines to: [1] understand the biology of the ecosystem; [2] communicate a credible plan to regulatory authorities; [3] reduce and relocate sampling locations to provide information on a watershed basis; [4] reduce the frequency of sampling to reflect the hydrology of the lake system; [5] conduct sediment and biological monitoring on a five-year cycle; and [6] develop triggers for reducing the program over time in response to results. The presentation will show how annual monitoring costs were immediately reduced by one-half and will also describe assessment triggers that will promote further cost reductions over time in response to environmental performance.

Using Benthic Invertebrate Communities as Indicators of Effects from a Municipal Wastewater Discharge. C. Larose[1], F. Landry[2] and T.C. Michelsen[3]. [1]Environmental Programs, Capital Regional District, Victoria, BC; [2]EVS Environment Consultants, North Vancouver, BC; [3]Avocet Consulting, Kenmore, WA.

A sediment monitoring program has been conducted at the Macaulay Point wastewater outfall in Victoria, British Columbia, since 1988 to assess the effects of wastewater discharges on the marine receiving environment. Benthic community and sediment chemistry analyses were conducted as part of this program in 1994 and 1997 from 20 sampling stations within 800 m of the outfall and at a reference station. Benthic community indices (i.e., species richness and polychaete abundance) as well as community structure were analyzed as a direct measure of potential effects from the discharge. Results from both 1994 and 1997 indicated an increase in polychaete abundance and a decrease in species richness within 200 m of the outfall in 1994 and 100 m of the outfall in 1997. These indices showed no statistical difference from the reference area for stations within 200-800 m. Sediment concentrations of different chemicals were also measured in both years and compared to sediment quality guidelines (SQGs) from the Canadian Council of Ministers of the Environment (CCME) and the Washington Department of Ecology. The accuracy of these guidelines in predicting effects on the biological community at Macaulay Point was measured by conducting a reliability analysis of each set of SQGs. The Washington State guidelines were strongly correlated with benthic effects near the outfall. Sediment chemistry concentrations, when compared to these guidelines, predicted the same pattern of effect as seen for the benthic community analysis (i.e., effects were generally isolated within a 100 m of the outfall). Site-specific, direct measurements of benthic communities at Macaulay Point are good indicators of wastewater discharge effects on the seafloor.

Weight-of-Evidence in EEM Benthos Assessments - What does it Really Mean? G.P. Thomas and N. Munteanu. G3 Consulting Ltd., Richmond, BC.

EEM investigations ascribe to the regulatory standards which stipulate the assessment of select endpoints of multi-components upon which weight-of-evidence analysis are used. Investigations often occur in complex environments where these components interact in differing ways to comprise a highly dynamic and variable receiving environment. When used properly, weight-of-evidence analysis may increase the ability of a researcher to distinguish and define effects. By examining different types of analytical assessments this paper elucidates the meaning and use of weight-of-evidence analysis in

benthos monitoring and provides examples of its ability to discern effects to natural benthic communities within complex systems. This paper describes weight-of-evidence analysis as relationship-linked and fully integrates component- and function-based analyses within spatial analysis of effects on the receiving environment. The paper describes the limitations of "component stacking", population-level endpoints, and indices to achieve ecological relevance. The importance of function-based and relationship-linked criteria (e.g., competition, predation, behaviour/feeding strategies, ecological niche) and use of relationship-linking models such as Pearson and Rosenber's SAB are described as part of weight-of-evidence analysis and good science.

Data Interpretation Guidance for Canada's National Environmental Effects Monitoring Program. R.B. Lowell[1], K. Hedley[2] and E. Porter[2]. [1]Environment Canada, National EEM Office and National Water Research Institute, Saskatoon, SK; [2]Environment Canada, National EEM Office, Hull, QC.

As part of Canada's National Environmental Effects Monitoring (EEM) Program, regulated pulp and paper mills and metal mines are/will be required to submit an interpretive report describing monitoring results. General guidance has been prepared on how to interpret this EEM data - specifically: [1] which effect endpoints to use; [2] the statistical (or other) approach to use for each endpoint to determine the presence or absence of an effect; and [3] the role of power analysis, α, β, and effect size in determining effects. A statistically significant change (relative to reference conditions) in any of the effect endpoints is to be considered an "effect" for the purposes of warranting possible follow-up action. Such an effect does not, however, necessarily indicate ecological, social, or economic significance sufficient to require corrective action. The endpoints are normally based on field monitoring data, although acceptable alternative methodologies are also available. Power analyses should be conducted both at the beginning of a study to determine required sampling effort and at the end of a study to determine whether the power that was actually achieved was sufficient to detect the effect size of interest. A key recommendation is to set $\alpha = \beta$ as a starting point for data interpretation. The data made available in the EEM program reports are currently being used as part of a national meta-analysis of pulp and paper mill effluent effects on aquatic biota.

What Happens After EEM Identifies an Effect. K. Hedley[1], K.R. Munkittrick[2,3] and A. Colodey[4]. [1]Environment Canada, National EEM Office, Hull, QC; [2]Environment Canada, National Water Research Institute, Burlington, ON; [3]Department of Biology, University of New Brunswick, Fredericton, NB; [4]Environment Canada, Pacific and Yukon Region, Vancouver, BC.

Recently, the Pulp and Paper EEM Requirements were redrafted as part of the process of amending the *Pulp and Paper Effluent Regulations*. Over the same period, the Metal Mining EEM program was developed. As a result of these activities occurring simultaneously, the EEM programs are now designed to meet the same objective, to evaluate the effects of effluents on fish, fish habitat and the use of fisheries resources. An effect is defined for both programs as a statistical difference in fish or benthic invertebrates between a site exposed to effluent and a reference site. From the perspective of the Federal Government, the EEM data will be used to assess the adequacy of the regulations in protecting fish, fish habitat and the use of fisheries resources. Environment Canada has worked with Fisheries and Oceans Canada over the past year to develop a guidance document outlining how the federal government will use the information provided by the EEM to assess the adequacy of the regulations, as well as how a determination regarding the need for enhanced protection will be made. The contents of the guidance document will be presented including why the guidance document is needed; when the guidance document will be implemented; how the adequacy of the regulations will be determined at a site-specific, regional, or national scale; when corrective measures will be considered; and, roles and responsibilities of stakeholders after EEM programs identify effects.

POSTER SESSION

Session Chair: T. Ellen

Oil and Gas Environmental Effects Monitoring

Offshore Hydrocarbon Development, Environmental Management and Environmental Effects Monitoring (EEM). K.K.J. Kim, D.L. Wallace, L.M. Fanning and A. Gauthier. Environment Canada, Environmental Protection Branch, Dartmouth, NS.

This poster provides an overview of the role of Pollution Prevention (P2), environmental management systems(EMS) and EEM in enhancing environmental management in the offshore oil and gas sector. Good environmental management can minimize impacts to the environment as well as reduce operating costs, financial risks and liabilities, improve productivity, increase profits and enhance corporate image. Good environmental management is facilitated by the establishment of an EMS and the adoption of a P2 approach.

Offshore Hydrocarbon Development, Aquatic Environmental Effects Monitoring (EEM). K.K.J. Kim[1], K.A. Coady[2] and W.R. Parker[3]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NS; [2]Environment Canada, Environmental Protection Branch, St. John's, NF; [3]Environment Canada, Environmental Protection Branch, Fredericton, NB.

Offshore oil and gas activities in Atlantic Canada have increased substantially over the last number of years. Operational discharges, including drilling muds, drill cuttings and produced water, have been known to cause environmental impacts in the marine environment. While these impacts may be localized and/or of limited duration, any environmental damage arising from offshore petroleum activities should be minimized. The poster focuses on the adequacies of the existing EEM programs, the use of EEM data and research needs as seen by Environment Canada.

Screening of Chemicals for Use Offshore at the Terra Nova Development. U. Williams[1] and M. Murdoch[2]. [1]Terra Nova Development, St. John's, NF; [2]Jacques Whitford Environment Ltd., St. John's, NF.

Chemicals used offshore may pose a risk to the people, facilities and the environment. Consistent with Terra Nova's commitment to environmental protection, it is Terra Nova's objective to select chemicals with the lowest risk to people and environment while balancing this with economic and efficiency considerations,and protection of assets. This presentation outlines the chemical selection process used by Terra Nova. This process includes health and safety checks with regulations, environmental discharge screening and risk assessment. Chemicals subject to this process include those used for drilling, production, vessel maintenance, subsea construction, laboratory, and some janitorial work. Chemicals planned for discharge to the marine environment are screened using guidelines developed by the Offshore Petroleum Boards for Newfoundland and Nova Scotia. These guidelines include checks against applicable Canadian legislation (e.g., *Canadian Environmental Protection Act, Pest Control Products Act*) and follow OSPARCOM steps for product review in the North Sea. The output of this process is an Approved Chemical List that links each chemical with specific conditions for use for Terra Nova. Chemicals are monitored for amount used and fate. This information may, in turn, be used to refine the EEM design and aid in EEM data interpretation.

Characterization of Natural Hydrocarbon Release from Oil Sands Deposits in Tributaries of the

Athabasca River Basin, Canada. J.V. Headley[1], M. Conly[2], L.C. Dickson[1], C. Akre[1] and K.M. Peru[1]. [1]Environment Canada, National Water Research Institute, Saskatoon, SK; [2]Environment Canada, Canadian Wildlife Services, Saskatoon, SK.

The Athabasca river basin covers a vast region of Western and Northern Canada, and flows through the Fort McMurray formation, an area rich in natural oilsands deposits. For future developments in the region, it is important to define base-line conditions and ascertain what contributions to river loadings of hydrocarbons are naturally derived, as opposed to arising from anthropogenic sources. For downstream sediment samples of tributaries where there was little or no mining activities, there was a preponderance of hydrocarbons, primarily PAHs, their C_{1-4} alkylated analogues, and biomarkers, relative to upstream samples. The relative compositions were diagnostic of a petrogenic origin, and provided a fingerprint of natural sources for integrating chemical data with monitoring programs. The latter included the characterization of biofilm samples, aquatic invertebrates, and sediments in northern rivers and tributaries. Mass spectrometry analyses of sediments, confirmed that the tributaries passing through the Fort McMurray oil sands regions contain significant levels of naturally derived hydrocarbons (2- 50 g/kg), particularly the Ells (50 g/kg), Mackay (8.4 g/kg), and Steepbank (7.1 g/kg). Monitoring activities should therefore focus on the tributaries draining through oil sands deposits, as opposed to the main-stem of the Athabasca River, where hydrocarbon levels are subject to dilution from high sedimentation rates.

The Effects of Oil Sands Derived Chemicals, Naphthenic Acids and Sodium Sulphate on Larval Fathead Minnows. A.J. Farwell and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

Surface mining and processing of the Athabasca oil sands deposits in northeastern Alberta, Canada results in the production of a significant quantity of process affected tailings water. The composition of the tailings water includes three major chemical stressors; naphthenic acids (NAs), salinity and polycyclic aromatic hydrocarbons (PAHs). Although the toxicological effects of a variety of PAHs are well documented, there is little information on the effects of NAs and the interactive effects of NAs and salinity on biota. This study is part of a larger project investigating the effects of NAs and salinity on a variety of different biota. The present study focuses on the interactive effects of salinity (sodium chloride and sodium sulfate) and NAs on the survival and growth of larval fathead minnows (*Pimephales promelas*). The NAs tested include commercially available NAs (cyclohexane carboxylic acid and abietic acid), in addition to the NA fraction extracted from tailings water (chemical composition unknown). The information obtained from this study is critical to the assessment of the cumulative effects of oil sands derived chemical mixtures.

Determining the Effects of Methylated Polycyclic Aromatic Hydrocarbons on the Development of Embryo/Larval Japanese Medaka (*Oryzias latipes*)**.** S.M. Rhodes, A.J. Farwell and D.G. Dixon. Department of Biology, University of Waterloo, Waterloo, ON.

The Athabasca oil sands (Alberta, Canada) represent one of the world's largest petroleum deposits. Present extraction procedures create large amounts of waste material (fine tailings) which are deposited in maturation ponds. During the maturation process, both naphthenic acids and polycyclic aromatic hydrocarbons (PAHs) are released. Analysis of tailings water shows higher concentration of methyl/alkyl PAHs compared to unsubstituted varieties. alkylated PAHs are not listed as USEPA environmental priority pollutants. Since little is known about the toxicological effects of the methylated/Alkylated PAHs, found in tailings water. This study focuses on determining the toxicological effects of a number of methyl substituted PAHs; including 9,10-dimethylanthracene, 3,6-dimethylphenanthrene, 4,6-dimethyldibenzothiophenene, and DMBA, on the development of

embryo/larval Japanese medaka (*Oryzias latipes*). It is anticipated that the response will be equivalent or greater than that of unsubstituted PAHs due to increased alkyl solubility. In addition, the ability of methylated PAHs to induce blue sac disease in embryo/larval medaka will be examined. Preliminary results demonstrate that methyl PAHs induce a significant toxicological response, which is equivalent or greater than that of the unsubstituted variety. Tests indicate higher instances of blue sac disease in exposed embryo/larva relative to controls.

Salinity Effects on the Toxicity of Naphthenic Acids to Rainbow Trout Cell Lines. L.E.J. Lee[1], K. Haberstroh[1], D.G. Dixon[2] and N.C. Bols[2]. [1]Department of Biology, Wilfrid Laurier University, Waterloo, ON; [2]Department of Biology, University of Waterloo, Waterloo, ON.

Reclamation of oil from the oil sands in Alberta has led to environmental concerns regarding its extraction procedures as large volumes of tailings are released into the environment. Three major chemical stressors have been identified within these tailings, naphthenic acids (NAs), salinity and polycyclic aromatic hydrocarbons (PAHs). Whereas the toxicity of PAHs has been widely documented, comparatively little is known about the effects of NAs and salinity to the biota, although acute toxicity has been reported in fish with NAs at concentrations found in fresh tailings water. NAs are complex mixtures of saturated carboxylic acids found at high levels in oil-sands tailings whose exact chemical composition is not known. The present study was designed to investigate the cytotoxic effects of NAs and to develop rapid cost-effective bioassays to quantify NA mixture toxicity. A gill and liver derived rainbow trout cell lines were examined for impairment in several cellular functions using a fluorescence microplate reader. Cell membrane integrity, mitochondrial activity and lysosomal function were monitored at various NA and salinity concentrations and time points. Salinity appeared to enhance the toxicity of NAs. This approach could provide a reference base for rapid assessment of NA toxicity as well as from its fractions and mixtures to biomonitor oil constituents and contribute to environmental risk assessment. (Supported by TSRI).

Analysis of Oxygenated Polycyclic Aromatic Hydrocarbons by Liquid Chromatography-Mass Chromatography with Comparison to Conventional Gas Chromatography-Mass Spectrometry. G.A. MacInnis, B.G. Brownlee, H.-B. Lee and T.E. Peart. Environment Canada, National Water Research Institute, Burlington, ON.

Oxygenated polycyclic aromatic hydrocarbons (OPAH) can be produced in the environment by metabolism or photooxidation of parent PAHs. OPAHs are of interest and concern because of their potentially genotoxic effects. The analysis of these polar compounds by gas chromatography-mass spectrometry (GC-MS) may require derivatization to overcome low volatility and/or thermal lability. Conjugated hydroxyl-PAHs (glucuronides, sulfates) need to be hydrolyzed either chemically or enzymatically before derivatization and analysis by GC-MS. Liquid chromatography-mass spectrometry (LC-MS) offers a more direct route for the analysis of OPAHs and their conjugated derivatives. In this preliminary study we are examining LC-MS with electrospray ionization (ESI) for the analysis of representative hydroxylated PAHs. The results are compared with those obtained from conventional GC-MS with derivatization.

The Influence of Produced Water on Natural Populations of Marine Bacteria. M.R. Anderson[1], R.B. Rivkin[2] and P. Warren[2]. [1]Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, St. John's, NF; [2]Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, NF.

ABSTRACT

Produced waters are a complex and variable mixture of inorganic and organic nutrients and contaminants. They are also the largest waste stream from oil and gas production activities. Models of effluent fate predict that they will be rapidly diluted and dispersed when discharged into the open ocean. Because of their complexity and variable composition however, it is difficult to predict the both short-term effects of produced waters on planktonic populations prior to dilution and the longer term effects of low level contaminant or nutrient additions to nutrient-limited marine systems. Here we report the results of experiments designed to assess the effect of produced water from Cohasset-Panuke on natural bacterial populations. Experiments used varying ratios of salinity adjusted produced water to filter-sterilized seawater (PW:FSW) inoculated with either naturally occuring North Atlantic bacteria or with *Vibrio* sp. isolated from the North Atlantic. *Vibrio* were inhibited by concentrations of PW between 4-28% but were slightly stimulated by lower (0.03 and 0.05%) concentrations over a 24 h period. The natural bacterial population was inhibited by PW additions (2-25%) during the first three days of the experiment. By the sixth and final day, all concentrations of PW had stimulated the natural population relative to unammended controls. These results suggest an initial inhibition followed enhanced growth that may result from a release from nutrient limitation after the bacterial community adapts to the produced water and/or as toxicity is decreased by chemical modification of the toxic elements.

INTRODUCTION

Produced water, the aqueous effluent generated during oil and gas production, is the single largest waste stream generated by offshore oil and gas operations (Neff *et al.* 1987). The Hibernia operation for example, is predicted to generate up to 1000 m^3 of produced water per hour. In most offshore facilities it is discharged directly into the ocean. It is a complex and variable mixture including formation water, injection water and various treating chemicals.

The chemical composition of produced waters is highly variable in time and space. They contain a variety of potentially toxic substances including hydrocarbons, heavy metals, radionuclides and chemical additives such as corrosion inhibitors and biocides (Stephenson 1992). They are usually highly saline and may contain significant amounts of hydrolysis metals (e.g., Fe, Mn, Al), ammonia and other inorganic nutrients. As a result, produced waters have the potential for both stimulatory and toxic effects. Standard bacterial bioassays (Microtox®) indicate toxic effects at concentrations ranging from 0.2-24% produced water (Din and Abu 1993) while mesocosm studies have shown a stimulatory effect on bacterial biomass at low (0.13-0.25%) concentrations (Gamble *et al.* 1987).

It is generally thought that the toxic effects of produced water discharges will be rapidly mitigated by dilution in the open ocean (Stephenson 1992). Nutrient additions however, have the potential to stimulate planktonic production at greater dilution factors and thus at greater distances from the discharge point. For example, the ammonia concentration in the produced waters from Cohasset Panuke are approximately 36 mg/L (K. Lee personal communication) and up to 250 mg/L at sites in the North Sea (Tibbetts *et al.* 1992) while inorganic nitrogen in offshore seawater is usually in the µg/L range in the spring and undetectable during the summer. Produced waters are also high in dissolved organic carbon and volatile fatty acids which can serve as substrates for enhanced bacterial growth. As a result, produced waters have the potential to cause increased planktonic production even at significant distances from their discharge point.

The objective of our study was to determine the effect of produced water on the natural bacteria from the Northwest Atlantic. To this end we incubated bacterioplankton from Logy Bay, Newfoundland with produced water from the Cohasset Panuke oil field (Fig. 1).

MATERIALS AND METHODS

Produced water was obtained from the Cohasset Panuke site near Sable Island off the coast of Nova Scotia (Fig.1). Natural populations of bacteria used in the experiments were obtained from Logy Bay, Newfoundland (Fig. 1). Logy Bay is situated on the north east coast of the Avalon Peninsula and receives water from the inshore branch of the Labrador Current. Bacterial dynamics in Logy Bay are similar to those in nearby offshore regions and Logy Bay populations may be considered representative of North Atlantic bacteria.

Fig. 1. Map showing the location of Logy Bay and the Cohasset Panuke site near Sable Island.

Prior to an experiment, the produced water was diluted with deionized water to reduce the salinity from 90 ppt to approximately 35 ppt. This was done to avoid causing osmotic stress to the natural bacterial population. Water from central Logy Bay (5 m depth) was filtered through a 1 µm filter to remove grazers. 75 mls were inoculated into 500 ml incubation flasks. Varying proportions of the diluted produced water and filter sterilized seawater were added to create a geometric progression of produced water concentrations (Table 1). The flasks were incubated in the dark at approximately ambient temperatures for the duration of the experiment (Table 1). Incubations were also performed using *Vibrio* sp. (isolated from the North Atlantic). Samples for bacterial enumeration were collected daily and preserved in 2% final

Table 1. Experimental conditions.

Bacteria	Percent produced water	Incubation temperature	Duration
Natural Population - May	0, 2, 5, 9, 15, 25	2°C	6 d
Natural Population - August	0, 0.03, 0.5, 4, 14, 29	14°C	6 d
Vibrio sp.	0, 0.03, 0.5, 4, 14, 29	10°C	6 d

concentration glutaraldehyde. Bacteria were stained with acridine orange and counted by epifluorescence microscopy (Hobbie *et al.* 1977). Bacterial production was assessed by ^{3}H thymidine and ^{14}C leucine (Rivkin and Anderson 1997) incorporation in short term incubations at intervals during the experiments. Nutrient limitation was assessed for the August experiment and for the *Vibrio* experiment by the addition of 10 µmol Glutamic Acid.

RESULTS AND DISCUSSION

Vibrio

Vibrio grew rapidly in seawater amended with 10 µmol Glutamic Acid but did not grow in unamended seawater (Table 2). *Vibrio* grew over the first day in the lowest concentrations of produced water (0.03 and 0.5%). Cell numbers followed patterns similar to the control for the remaining 5 d.

Table 2. Growth rates of *Vibrio* sp.

Experimental conditions	Growth rate (d^{-1})
Control – 0.2 µm filtered seawater	0
+ 10 µmol Glutamic Acid	1.42
+ produced water	0-0.48 (initial 24 h) ~ 0 (day 2-6)

These results suggest that *Vibrio* sp. is severely nutrient limited in unamended seawater and that while the organic nutrients in the produced water are sufficient to stimulate short term growth this is countered by toxic effects at higher concentrations.

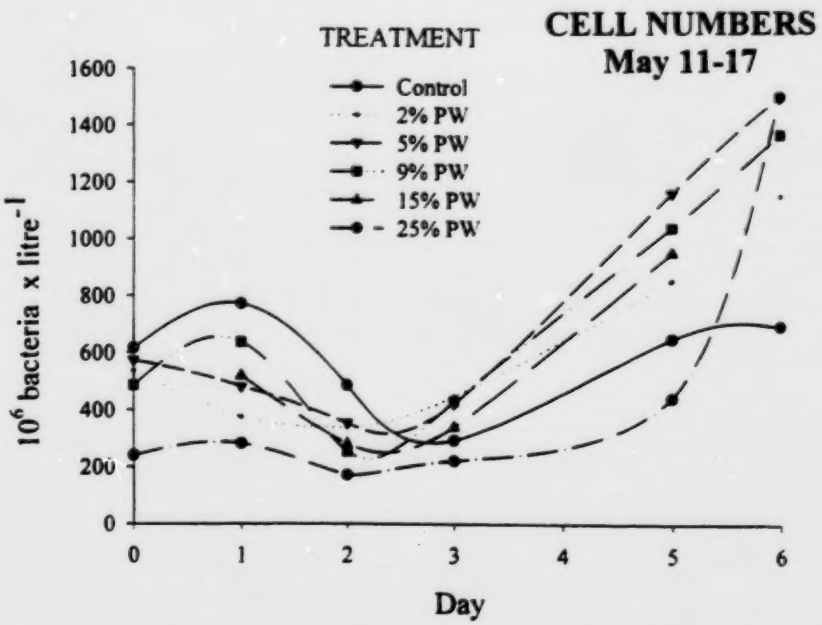

Fig. 2. Time dependent changes in cell numbers of North Atlantic bacteria incubated with differing concentrations of produced water from Cohasset Panuke. The treatments are % produced water (PW) added.

In May, even the lowest concentrations of produced water inhibited bacterial growth over the short term (2 d). However, by the sixth day of the experiments, bacterial numbers at all concentrations of produced water exceeded the controls (Fig. 2). These results suggest an initial toxicity followed by a release from nutrient limitation after the bacterial community adapts to the produced water and/or as toxicity is decreased by chemical modification.

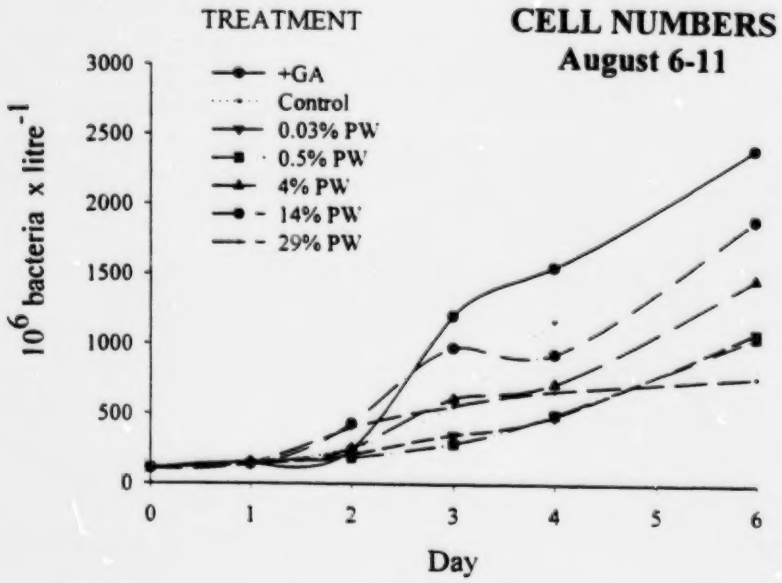

Fig. 3. Time dependent changes in cell numbers of North Atlantic bacteria incubated with differing concentrations of produced water from Cohasset Panuke. The treatments are % produced water (PW) added. GA represents the 10 µmol Glutamic Acid addition.

Table 3. Growth rates for bacteria from the North Atlantic in August incubated with differing concentrations of produced water from Cohasset Panuke.

Treatment	Growth rates (d^{-1})			Timing of maximum growth
	6 d aver.	minimum	maximum	
Control	0.48	0.23	0.78	Day 3
+ 10µmol GA	0.52	0.22	1.62	Day 3
+ 0.03% PW	0.37	0.14	0.45	Day 4
+ 0.5% PW	0.37	0.23	0.50	Day 4
+ 4% PW	0.43	0.16	0.90	Day 3
+ 14% PW	0.47	-0.07	1.13	Day 2
+ 29% PW	0.33	0.08	1.01	Day 2

GA = Glutamic Acid; PW = Produced Water

In August, the bacterial populations exhibited nutrient limitation that was not completely alleviated by the produced water additions (Fig. 3). Over the short term (2 d), the highest concentrations of

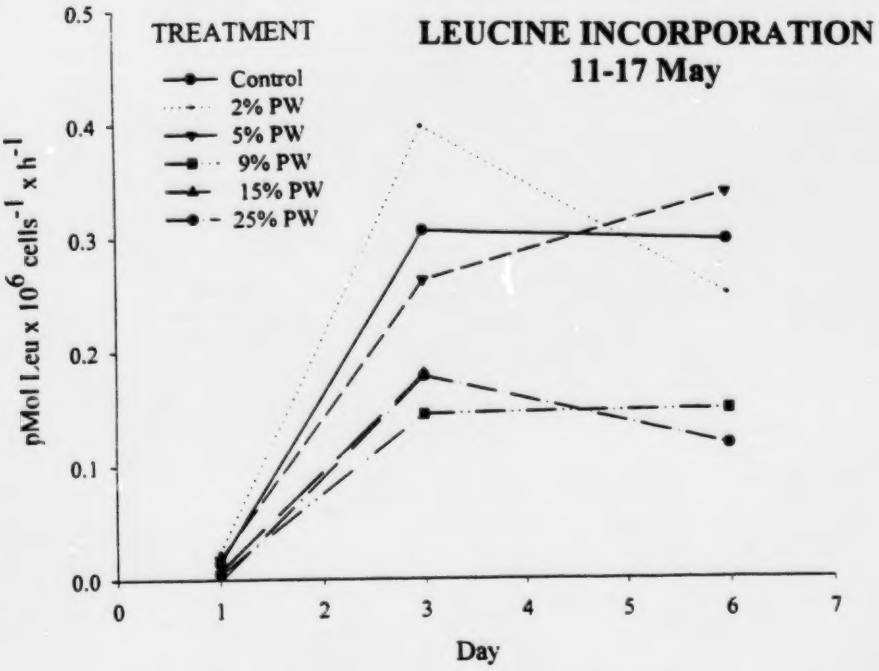

Fig. 4. ^{14}C Leucine incorporation by North Atlantic bacteria as a function of produced water concentration (%PW) on days 1, 3 and 6 of the May experiment.

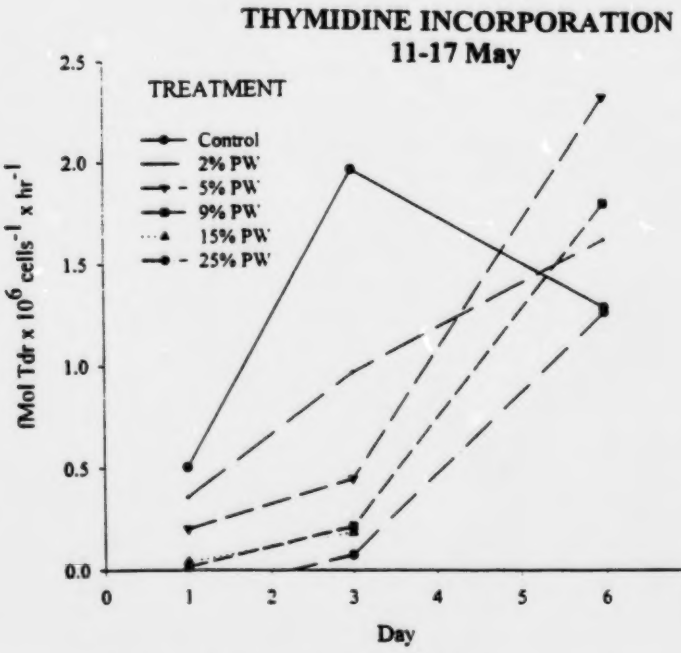

Fig. 5. ^{3}H Thymidine incorporation by North Atlantic bacteria as a function of produced water concentration (%PW) on days 1, 3 and 6 of the May experiment.

produced water increased bacterial growth rates (Table 3) however, by the sixth day the intermediate concentrations had cell numbers equal to the control. Bacterial numbers in the lowest and highest concentrations of produced water were 50-60% lower than the control (Fig. 3).

Bacterial Production

Bacterial uptake of thymidine and leucine showed varying trends to increasing concentrations of produced water. By day 3 of the May experiment, leucine incorporation was greatest for the 2% PW treatment while all other concentrations were below the control (Fig. 4). By day 6, leucine incorporation rates were similar for the two lowest treatments and the control. Higher concentrations of produced water continued to show reduced rates of leucine incorporation. Thymidine incorporation rates for all treatments were reduced compared to the control on day 3 (Fig. 5). By day six all PW treatments had greater rates of incorporation than the control except for the highest concentration (25% PW). These results suggest that the influence of produced water on substrate incorporation will vary with substrate type and that care must be taken in interpreting the results of experiments to determine bacterial productivity.

CONCLUSIONS

The direction, magnitude and timing of the response of natural North Atlantic bacteria to produced water additions varies over the season. Cultured species such as *Vibrio* do not show similar response to produced water as naturally occurring bacteria. Standardized tests using such species cannot be considered representative of the natural populations. Experiments with naturally occurring bacteria indicate the potential for produced water to inhibit bacterial growth over the short term and/or at high concentrations with the potential to enhance growth at lower concentrations over the longer term. Methods for the measurement of the influence of produced water on bacterial productivity need to be further investigated given the differential response of substrate incorporation rates to produced water concentrations.

ACKNOWLEDGEMENTS

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Cumulative Effects Monitoring

Enrichment of Canadian Ecosystems from Nutrient Additions: Lakes, Rivers, Wetlands, and Coastal Waters. E.S. Roberts[1], P.A. Chambers[2], R.A. Kent[1], C. Gagnon[3] and M.N. Charlton[4]. [1]Environment Canada, Guidelines and Standards Division, Ottawa, ON; [2]Environment Canada, National Hydrology Research Institute, Saskatoon, SK; [3] Environment Canada, CSL Chimie de l'environnement, Montreal, QC; [4]Environment Canada, National Water Research Institute, Burlington, ON.

In a 1994 review of the *Canadian Environmental Protection Act* (CEPA), the Parliamentary Standing Committee on Environment and Sustainable Development identified several issues related to nutrients and their environmental impact and management that required further study and perhaps, even legislation. This ongoing project is the response by the Government of Canada to quantitatively and qualitatively determine the impacts of nutrients from various sources (i.e., atmospheric, agriculture, municipal, and industrial) on the Canadian environment. Nutrients such as nitrogen and phosphorus are essential to plant growth; however, in aquatic ecosystems, excessive nutrients often leave the water quality unacceptable for the protection of the habitat and health of animals and for the recreational use by humans. Enhanced plant growth is the primary consequence of nutrient addition for all aquatic ecosystems (lakes, rivers, wetlands and coastal waters). The secondary consequences (e.g., changes in floral and faunal diversity, effects on nutrient cycling, impacts on adjacent ecosystems) differ amongst ecosystems. Canadian case studies (i.e., Lake Erie, ON; Cootes Paradise Marsh, ON; Bay of Fundy, NS) illustrate the general impacts of nutrients on these ecosystems. The findings from these case studies will be used to make recommendations to assist the environmental management of nutrients in the environment.

Using Measures of Sub-Lethal Stress in *Mytilus* spp. for Contaminant Monitoring in the Gulf of Maine and Bay of Fundy. A.E. Monette[1] and P.G. Wells[1,2]. [1]School for Resource and Environmental Studies, Dalhousie University, Halifax, NS; [2]Environment Canada, Environmental Conservation Branch, Dartmouth, NS.

The Gulf of Maine Council on the Marine Environment's Gulfwatch program currently uses *Mytilus* spp. body burdens and tissue concentrations of contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, chlorinated pesticides and trace metals) as indicators of marine habitat exposure. Such data from mussels are intended to reflect ambient contaminant concentrations and contribute to assessments of the overall health of the Gulf of Maine and Bay of Fundy ecosystem. In addition to contaminant measures, Gulfwatch also employs biological measures, for example, mussel shell growth, condition index and gonad index. To enhance this biomonitoring program and better demonstrate an ecotoxicological connection, practical measures of sub-lethal stress in *Mytilus*

could be added to the current monitoring approach. Several promising measures of sub-lethal stress using *Mytilus* spp. were evaluated based on criteria chosen to emphasize potential relevance and practicality within the Gulfwatch monitoring framework, and also within the context of developing a Framework for Cooperative Marine Monitoring (FCMM). From this evaluation, three measures of sub-lethal stress in mussels, including haemolymph lysosomal membrane neutral red retention, flow cytometric determination of DNA damage, and embryo-larval development/mortality assays, were identified as promising for use by Gulfwatch. By expanding the Gulfwatch monitoring approach to include additional endpoints with mussels and eventually other species, an assessment of both exposure (tissue burdens and concentrations) and effects (measures of sub-lethal stress) could be used in ecological risk management within the Gulf of Maine and Bay of Fundy.

DNA Adducts Levels, Hepatocyte Proliferation Rates and Ethoxyresorufin O-Deethylase Activity in Atlantic Tomcod from the St. Lawrence Estuary. C.M. Couillard[1], R. Shah[2], G. Poirier[2] and B. Légaré[1]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [2]CHUL Research Center, Ste-Foy, QC.

Neoplastic lesions of unknown origin are found in the liver of Atlantic tomcod in the St. Lawrence Estuary. A three year study was initiated in 1999 to identify the major mechanisms leading to carcinogenesis in these fish exposed to complex mixtures of contaminants. The effect of age on various bioindicators was investigated in females Atlantic tomcod captured at La Baleine, Ile-aux-Coudres, QC. Hepatic DNA adducts were measured by ^{32}P-postlabeling in parallel to hepatocyte proliferation rates, assessed by immunohistochemical staining of the proliferating cell nuclear antigen, and to hepatocyte nuclear size, measured with an image analysing system. The activity of the biotransforming enzyme ethoxyresorufin O-deethylase (EROD) was also assessed. The levels of DNA adducts increased linearly with fish age suggesting accumulation of persistent adducts or alteration of the detoxifying capacities in older animals. The percentages of hepatocytes with large nuclei also increased with age and may be indicative of cytotoxicity. The hepatocyte proliferation rates and the activity of EROD were higher in one year old fish compared to older fish. In older fish, the variability of hepatocyte proliferation rates within fish, but not the activity of EROD, was positively correlated to the levels of DNA adducts. Thus, fish age influenced the responses of all bioindicators considered.

Are Vertebral Malformations Associated with Toxaphene Contamination in Atlantic Tomcod from the St. Lawrence Estuary? C.M. Couillard[1], B. Gouteux[2], M. Lebeuf[1] and H. Dionne[2]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [2]ISMER, Rimouski, QC.

Toxaphene, a persistent organic pollutant, found in the tissues of aquatic organisms from the St. Lawrence Estuary, is known to induce vertebral malformations in various species of fish. The possible association between the presence of vertebral malformations and the degree of contamination with toxaphene and other persistent organic contaminants was investigated in Atlantic tomcod from La Baleine, Ile-aux-Coudres, QC, in the St. Lawrence Estuary. To evaluate the prevalence of vertebral malformations, 129 tomcods were x-rayed. Nearly 20% of the tomcods had vertebral malformations, mostly vertebral compressions. The vertebral density and the levels of toxaphene, chlorinated pesticides and polychlorinated biphenyls in the liver were compared between fish affected with vertebral malformations and fish of similar age and length without vertebral malformations. The density of vertebrae was reduced in fish affected with vertebral malformations suggesting biochemical alterations in the vertebral body. Levels of contamination with toxaphene and other contaminants exhibited marked inter-individual variations but did not differ significantly between malformed and control fish. Malformed fish exhibited higher variability in the levels of contaminants than control fish as a consequence of severe depletion of liver lipids in the most severely affected individuals. Further

studies will evaluate the effects of toxaphene on the vertebrae of marine fish embryos.

Habitat Degradation and Cumulative Effects on Fish Health in St. John's Harbor. B. French, W. Melvin, M. Dawe and A. Mathieu. OCEANS Ltd., St. John's, NF.

Coastal zones often receive large quantities of contaminants posing special risks to fish and fish habitat. St. John's Harbor has been used as a disposal site for raw sewage, storm water overflow, and effluents from light industry for a number of years. Studies have been carried out to establish if Harbor habitat has been degraded to a sufficient degree to adversely affect the health of winter flounder, a commercial species commonly found in inshore waters. A variety of health effects were assessed in mature female fish captured within and just outside the Harbor. Fin erosion was present in the majority of fish within the Harbor, but absent in fish from outside the Harbor. Mixed function oxygenase activity (measured as EROD) was significantly elevated in flounder taken from the Harbor. Gill edema was also more severe in fish captured within the Harbor. Likewise, an increase in the percentage of neutrophils was found in flounder taken within the Harbor. Liver pathology is often indicative of more long-term exposure to deleterious contaminants. In this regard it is worth noting that four types of hepatic lesions associated with aquatic pollution (neoplasia, hydropic vacuolation, macrophage aggregation and hepatocellular necrosis) were identified in fish taken within as well as outside the Harbor. Overall, the results clearly demonstrated that the habitat of St. John's Harbor and possibly nearby adjacent waters is degraded to a sufficient degree to adversely affect the health of fish. The study also supports use of pathological/histopathological effects in fish for assessing habitat degradation. (Study supported by the Atlantic Coastal Action Program).

Using Bioindicators *Mytilus edulis* and *M. trossulus* to Determine the Distribution and Transport of Toxic Metals in St. John's Harbour, NF. N.E. Leawood[1] and P.J. Sylvester[2]. [1]Department of Environmental Science[1], Memorial University of Newfoundland, St. John's, NF; [2]Department of Earth Sciences, Memorial University of Newfoundland, St. John's, NF.

This study investigates the fate and transport of metals entering St. John's Harbour water by analyzing the metal concentrations of indigenous mussels using inductively coupled plasma mass spectrometry (ICP-MS). Preliminary results of samples from seven sites, ranging from the inner harbour to the outer bay area, indicate no significant systematic geographic variations in concentration for Zn and As. The average concentration in parts per million ranged from 79-155 for Zn and 5.6-8.0 for As. These values of Zn and As do not exceed the high end concentration levels set by the US Mussel Watch Project of 200 mg/kg for Zn and 17 mg/kg for As. Also, the measured Zn and As concentrations fall between the 50th and 85th percentiles of data published by Cantillo in 1998, where the US and France Mussel Watch programs were compared with the Worldwide Study. Although no data have been acquired to date, other metals such as Cu, Co, Cr and Pb will be analysed. Concentrations of Hg, Cd, Sn, Ag and Mo will be determined using the technique of isotope dilution with ICP-MS. Also, stable isotope ratios will be used tc indicate sources of Pb contamination found in the mussels.

Temporal Trends in the Bioaccumulation of Organochlorines in a Sub-Arctic Food Web: Continuing Survey of Lake Laberge, Yukon Territory. M.J. Ryan[1,2], G. Stern[2] and W.L. Lockhart[2]. [1]Department of Soil Sciences, University of Manitoba, Winnipeg, MB; [2]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB.

Organochlorine contaminant (OC) levels in Lake Laberge lake trout have dropped up to 4 fold over the five year period from 1993-1998. In comparing OC levels in lake trout from Lake Laberge to trout from other Yukon lakes (1998, 1999 data only) and after accounting for lipid as a variable, levels of

OC in the Laberge trout no longer predominate as they had in the early 1990s. Concentrations of the more lipophilic contaminants such as toxaphene (sum of chlorinated bornanes or ΣCHB) and ΣPCB were highest in lake trout from Lake Kusawa while lake trout from Atlin Lake had the highest concentrations of the less lipophilic (ΣHCH and ΣCBz) OCs. A corresponding decline in organochlorine contaminant levels was observed in Laberge burbot livers. ΣCHB and ΣDDT lipid-adjusted concentrations decreased in the burbot liver by a factor of 3.5 and 4.3, respectively, over a nine-year period from 1990-1999. It is hypothesized that the decline in OC concentrations is linked to the increasing trout population in Lake Laberge following the shut down of the commercial and subsistence fisheries in 1991.

Developing an ASTM Standard Guide for Conducting In-situ Field Bioassays with Marine, Estuarine and Freshwater Bivalves. S.M. Salazar and M.H. Salazar, Applied Biomonitoring, Kirkland, WA.

We have been working through the American Society for Testing and Materials (ASTM) in the US since 1997 to develop a Standard Guide for conducting field studies with caged bivalves. We have conducted 40 bivalve transplant studies with over 50,000 animals and 15 different species (Fig. 1). These in-situ field bioassays have been conducted in marine, estuarine, and freshwater environments from the intertidal zone to a depth of 70 meters. This approach has been used to evaluate surface water quality, effluents, and sediment. In addition to our work, we identified from literature reviews approximately 300 other documented bivalve transplant studies (Table 1). Approximately 200 of these have been conducted in the marine environment and about 50% of those (~100) on *Mytilus* spp. Approximately 100 studies have been conducted in freshwater environments and of those, approximately 20% (~20) on *Elliptio* spp. and another 20% (~20) on *Corbicula* spp. This approach must be standardized to increase its utility.

Fig. 1. Transplant studies conducted by Salazar and Salazar.

Bivalves are suitable surrogate test organisms because of their ability to integrate chemicals in their tissues and utilize multiple exposure pathways such as water and food. Bivalves have been shown to be as sensitive or more sensitive than commonly used laboratory test species (Table 1). *In-situ* transplant studies with caged bivalves are particularly useful in areas where resident species are absent. Sublethal effects endpoints such as growth provide a continuum of responses and greater sensitivity than just measuring survival. Reduced growth rates in bivalves have been associated with population effects. Therefore, growth measurements have environmental significance as well as being relatively easy and inexpensive measurements to make. The proposed methodology enables simultaneous measurement of exposure and effects. It is important to quantify exposure because

Table 1. Relative sensitivity of bivalves, freshwater (F) and marine (M) species.

Bivalve Species	Species Compared	Exposure	Endpoint	Sensitivity
Anodonta grandis (F) (Giant Floater)	Daphnia, fathead minnow, rainbow trout	Municipal effluent	LD^{50}	Equal
Anodonta imbecilis (F) (Paper Pondshell)	Daphnia mill effluent	Pulp & paper mortality	10d vs 7 d	More
Anodonta imbecilis (F) (Paper Pondshell)	Daphnia, midge, fathead minnow	Metals	7 d mortality	Equal
Musculium transversum (F) (Fingernail Clam)	17 different species	Ammonia	20 d mortality	More than 16
Mercenaria mercenaria (M)	Amphipods, Microtox®	Sediment	7 d growth, 10 d mortality	More
Mulinia lateralis (M)	Amphipod	Sediment	7 d growth, 10 d mortality	More
Mytilus galloprovincialis (M)	Amphipod	In-situ water column	84 d growth 10 d mortality	More, [tissue TBT]

Caged *Mercenaria* more sensitive than lab *Mercenaria*

Table 2. Partial list of bivalves used in transplant studies for monitoring marine and freshwater environments. Many species can be used in estuarine environments due to their ability to tolerate a wide range of salinities. Asterisk (*) indicates species used in studies with compartmentalized cages; most other species have been deployed in non-compartmentalized cages, although a few benthic studies did not employ cages of any kind.

Marine Bivalve Test Species

Mussels	Oysters	Clams	Scallops
Arca zebra	*Crassostrea angulata*	*Anadara granosa*	*Aequipecten opercularis*
Modiolus modiolus	*Crassostrea gigas*	*Cardium edule*	*Amusium japonicum*
Mytilopsis sallei	*Crassostrea japonica*	*Crastoderma edule*	*Amusium pleuronectes*
*Mytilus edulis**	*Crassostrea rhizophorae*	*Chione stutuchburyi*	*Argopecten irradians*
*Mytilus galloprovincialis**	*Crassostrea virginica**	*Circenita callipyga*	*Argopecten purpuratus*
*Mytilus californianus**	*Ostrea edulis*	*Macoma balthica*	*Chlamys islandica*
*Mytilus trossulus**	*Ostrea angasi*	*Gomphina melanaegis*	*Chlamys varia*
Perna perna	*Ostrea lurida*	*Macoma inquinata*	*Crassodoma gigantea*
*Perna viridis**	*Perna sterna*	*Macoma nasuta**	*Hinnites multirugosus*
Pinna bicolor	*Saccostrea commercialis*	*Mercenaria mercenaria*	*Lima hians*
		Meretrix lamarchi	*Pecten maximus*
		Mya arenaria	Placopecten magellanicus

Table 2. Continued

		Panopea abrupta *Scrobicularia plana* *Spisula solidissima* *Venerupis japonica* *Venerupis staminea*	
10 spp; 6 genera	10 spp	17 spp	12 spp

Freshwater Bivalve Test Species

Mussels		Clams/Cockles
Actinonaias ligamentina	*Lampsilis higginsi*	*Anadara trapezium*
Actinonaias pectorosa	*Lampsilis orbiculata*	*Corbicula fluminea**
Amblema plicata	*Lampsilis powelli*	*Corbicula japonica*
Amblema perplicata	*Lampsilis radiata*	*Corbicula manilensis*
Anodonta anatina	*Lampsilis ventricosa*	*Musculium transversum*
Anodonta cygnea	*Lemiox rimuosus*	*Rangia cuneata*
Anodonta grandis	*Margaritifera falcata*	*Sphaerium striatinum*
Anodonta implicata	*Medionidus conradicus*	*Sphaerium simile**
Anodonta kennerlyi	*Proptera alata*	
Anodonta piscinalis	*Proptera capax*	
Anodontites trapesialis	*Pyganodon grandis**	
*Dreissena polymorpha**	*Quadrula quadrula*	
*Elliptio complanata**	*Unio pictorum*	
Epiblasma torulosa rangiana	*Villosa nebulosa*	
Epioblasma triquetra	*Villosa vanuxemensis*	
Fusconia subrotunda	*Westralunio carteri*	
Hydridella menziesi		
33 spp; 19 genera		8 spp

Summary of bivalve transplants: About 300 documented bivalve transplant studies; 200 marine studies, 50% = *Mytilus* spp.; 100 freshwater studies; 20% = *Elliptio* spp. & 20% = *Corbicula* spp. List does not include 19 marine and 1 freshwater aquaculture species .

bioaccumulation is the link between environment and organism, allowing associations between the lab and the field and other monitoring approaches to be made. Caged bivalves facilitate quantification of exposure and effects over space and time in almost any aquatic environment. Our recommended use of compartmentalized cages enables multiple measurements to be made on the same individuals and optimizes detecting differences among stations and establishing meaningful gradients of exposure and effects. The results can be used to identify gradients of contamination as well as establishing meaningful mixing zones based on biologically available chemicals.

The draft standard guide explains the use of caged bivalves to evaluate pathways of exposure and to establish bioaccumulation links. Documentation is also provided for species that have been used in transplant experiments (Table 2) and tolerance limits for commonly used species to selected water quality parameters (Table 3). Examples will also be provided to illustrate various deployment methods, compartmentalized cages for repetitive measurements on the same individuals, and a digital measurement and recording system. The purpose of the guide is not only to standardize the method, but to assist others in proper application of the method.

Table 3. Temperature (°C) and salinity (parts-per-thousand) tolerance limits for selected bivalve species. Months when spawning may occur and species distribution are also shown.

Species	Temperature Range	Salinity Range	Spawning Season	Distribution
Argopecten irradians (Bay scallop)	>7 < 30	>14 - 28	Mid-Atlantic: mid-April through early September; NY: June & July; NC and FL: Aug & Dec	Atlantic coast; Cape Cod to Gulf of Mexico
Crassostrea gigas (Pacific oyster)	4 - 24	25 - 35	July to August	Pacific coast; Pacific Northwest
Crassostrea virginica (Eastern oyster)	-2 - 36	5 - 32	Gulf of Mexico: Apr-Oct; Malpeque Bay, PEI: Jul-Aug; Bideford River Estuary, PEI: July	Gulf of Mexico to Cape Cod
Macoma balthica (Baltic Clam)	-2 - 23	5 - 30	June - August (Europe) July - September (US)	Greenland to France; Baltic & Wadden Seas; UK; N. CAN to Chesapeake; AK to SF Bay
Mercenaria mercenaria (Hard clam)	<0 - 35	12 35	Mar-Nov, depending on latitude & temperature. Peaks in July	Atlantic and Gulf coasts; abundant MA to VA
Mya arenaria (Soft-shell clam)	-1.7 - 32	10 - 32	Jun-Sep; once/yr north of Cape Cod, twice/yr south of Cape Cod	Atlantic coast from Labrador to SC; less in FL; AK & CA
Mytilus californianus (California mussel)	7 - 28	25 - 33	Continuous throughout year; peaks in Jul & Dec	AK to southern CA
Mytilus edulis (Blue mussel)	0 - 27	5 - 33	Differs between populations; some low-level throughout year; first in early summer, second in the fall	Atlantic coast, from Labrador to Cape Hatteras, NC
Mytilus galloprivincialis (Mediterranean mussel)	8 - 25	10 - 33	Similar to *M. edulis*, but several weeks later when temperature is maximum	Mediterranean, Europe, Atlantic France & British Isles, Japan, E. China to Korea, Australia, S. Africa; S. CA to OR
Mytilus trossulus (Pacific blue mussel)	0 - 29	4 - 33	July to September	Baltic Sea; W. Coast - Central CA to AK; E. Coast - Canadian Maritimes
Ostrea lurida (Olympia oyster)	6 - 20	NA - 25	Spring to fall; peaks in spring in south, mid-summer in mid-range and north	SE AK to Baja California
Protothaca staminea (Littleneck clam)	0 - 25	20 - 32	BC, Canada - January to March; AK - mid-July; southern CA - June.	Aleutian Islands, AK to Cape San Lucas, Baja California
Venerupis japonica (Manila clam)	13 - 21	24 - 31	Washington: once/yr May-Sep, peaks in Jun/Jul	British Columbia to CA
Corbicula fluminea (Asian clam)	2 - 25	0 - 5	May be continuous, usually twice/yr: spring/early summer; later summer	All west, gulf, and east coastal US to DE River; NM; OH & MS River systems
Dreissena polymorpha (Zebra mussel)	<0 - 35	0 - 6	May to Sept	CAN & NE US; Great Lakes, St. Lawrence River; MS, OH, IL & TN River drainages; NY Canals, Hudson River, Finger Lakes
Elliptio complanata (Eastern Elliptio)	0 - 30	0 - 3	Most June to July; Some May to Sept	Gulf St. Lawrence to GA; Great Lakes, except Lake Michigan & Lake Erie
Pyganodon (Anodonta) grandis (Floater mussel)	0 - 30 0 - 3	Most April to May; Some to late August	CAN Interior & St. Lawrence R. dainage; Hudson Bay, MI & MO R. drainages; NM, CO, TX, Mex	
Rangia cuneata (Atlantic Rangia)	8 - 32	0 < 19	VA: Early April to summer; FL: July - Nov; LA: Mar-May & late summer to Nov; Mexico: Feb-Jun & Sept to Nov	Gulf of Mexico coast from NW FL to Campeche, Mexico; along Atlantic coast to NJ

= marine
= freshwater

What Japanese Fish Can Tell Us About Prince Edward Island Streams. K. Teather[1], E. Duffy[1] and M.A. Gray[2]. Department of Biology, University of Prince Edward Island, Charlottetown, PE; [2]Department of Biology, University of New Brunswick, Fredericton, NB.

On Prince Edward Island, where approximately 50% of land is devoted to agriculture, there is substantial concern over the potential effects of pesticide runoff into aquatic communities. In two separate experiments we exposed Japanese medaka (*Oryzias latipes*) to sediments and water from island streams (selected to represent areas of varying agricultural intensity) to assess developmental responses to agricultural activity. In the first experiment, we compared the toxicity (as measured by embryonic development) of sediments from streams within areas of high, medium and low agricultural activity. Survivorship and swim bladder inflation were significantly lower in embryos exposed to sediments from areas of high agricultural intensity. In the second experiment, we compared relative toxicity of water and sediment within streams. Exposure to water samples resulted in higher embryonic mortality, smaller hatchling size and a higher number of skeletal deformities. The more detrimental effects of exposure to water samples may have been due to [a] higher toxicity of water than sediments, or [b] differences in exposure solution preparation. Regardless, these preliminary results suggest that medaka embryolarval bioassays may be a useful tool in assessing agricultural impact on aquatic communities.

Ecological Risk Assessment

Identifying Uncertainty in the Exposure Assessment of New Substances in Canada and Recent Developments to Address Exposure Uncertainty. E. Postlethwaite[1], M. Bonnell[2] and A. Atkinson[1]. [1]Environment Canada, Hull, QC; [2]Bonnell Environmental Consulting, Ottawa, ON.

Recently the New Substances Division (NSD) of Environment Canada undertook an examination of the uncertainties associated with the exposure assessment process used to estimate releases and environmental concentrations of new substances in Canada. The purpose of this study was to identify specific components of the exposure assessment process that contribute a high degree of uncertainty due to large extrapolations, assumptions or data gaps. These components were targeted for further development within the bounds of a screening level of assessment in order to reduce the overall uncertainty in the exposure assessment. Significant uncertainties were associated with: [1] the scenarios used to calculate the release of substances from industrial plants for specific and default uses; [2] the partitioning and removal of substances in Canadian wastewater treatment plants; [3] the dilution approaches used to calculate aquatic concentrations of new substances; and [4] the use of a one compartment model (aquatic) regardless of the partitioning of the substance. The results of the uncertainty examination resulted in the development of a strategy for improved exposure assessment of new substances. Under this strategy, the NSD has begun the Canadian validation of available chemical plant release scenarios, has begun adapting the USEPA's PDM4 model for Canada, has initiated the updating of the widely used STP model, has begun development of a temporal and spatial aquatic fate and transport model (CHEMSIMM), and has initiated the development of a screening level multi-media exposure assessment framework. These initiatives are the subject of other presentations at this meeting.

A Framework for the Ecological Risk Assessment of New Substances in Canada. D. Porter[1], E. Karalis[1], A.J. Atkinson[1], M. Bonnell[2] and D. Moore[3]. [1]Environment Canada, Hull, QC; [2]Bonnell Environmental, Ottawa, ON; [3]The Cadmus Group, Inc., Ottawa, ON.

Environment Canada is responsible for the ecological risk assessment (ERA) of new substances introduced into Canada and follows a standardized approach in conducting assessments. To illustrate

the assessment process to stakeholders and other interested parties, a framework was developed based on international risk assessment frameworks, with appropriate modifications. The New Substances Framework consists of five components including [1] Problem Formulation; [2] Exposure Assessment; [3] Effects Assessment; [4] Risk Characterization, and [5] Risk Management. The Problem Formulation phase is critical in the development and direction of the ERA. The Exposure Assessment phase involves estimating the Predicted Environmental Concentration (PEC) of a substance to which organisms will be exposed. During the Effects Assessment phase, the type and nature of effect(s) are characterized based on data generated from toxicity tests and other available sources. These data are used to calculate a concentration of concern (CC) that is compared to the assessment endpoint defined in the Problem Formulation phase. The Risk Characterization phase incorporates information from the Exposure Assessment and Effects Assessment phases (i.e., the PEC and CC) to calculate a risk quotient(s) for the introduction of a new substance into Canadian commerce. The Risk Management phase involves risk reporting, risk communication, presentation of risk reduction strategies and consideration of monitoring and enforcement measures.

Modifications of the Fugacity-based STP (Sewage Treatment Plant) Model for New Substance's Exposure Assessment in Canada. N.D. Domey and A.J. Atkinson. Environment Canada, Hull, QC.

Recently, the New Substances Division (NSD) of Environment Canada undertook an examination of the uncertainties associated with the exposure assessment process used to estimate releases and environmental concentrations of new substances in Canada. The results of the uncertainty examination resulted in the development of a strategy for improved exposure assessment of new substances. Under this strategy, the NSD recognized that further development of the STP model would increase the accuracy of removal predictions for new substances in Canada. It was identified that if the STP model could better address ionized substances, accommodate more physical-chemical properties of substances (e.g., Koc, biodegradation) and have an expanded suite of default treatment plants, a reduction in uncertainty would result. Probably the greatest utility of modified STP model for new substance assessment is the ability to consider the range of treatment efficiencies that could exist in various types of treatment plants across Canada. This is important for new substances once they become eligible for listing on the Domestic Substances List (i.e. the Canadian inventory of existing commercial substances), at which point the substance can be used for any use anywhere in Canada. Accordingly, an expanded suite of default treatment facilities (and their associated parameters) is needed in order to consider removal efficiencies across Canada based on the range of treatment facilities likely to exist in Canada. Modifications made, by NSD, of the widely used STP model will be the subject of this presentation.

An Ecological Risk Assessment of Lake Saint-Louis, Quebec: Multiple Toxicity of Copper and Zinc to Spawning Success in Fathead Minnow and Bluegill Sunfish. L.E. Tavera Mendoza[1], S.M. Ruby[1], P. Brousseau[2] and M. Fournier[2]. [1]Department of Biology, Concordia University, Montreal, QC; [2]INRS-Institut Armand-Frappier, Montreal, QC.

A risk assessment of the heavy metals Cu and Zn to fish indigenous to Lake Saint-Louis, Quebec (45, 50 N; 71, 20 W) was conducted. The assessment examined sublethal effects of Cu and Zn on reproduction through reduced spawning in fathead minnow (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*) and estimated the likelihood of risk from Cu and Zn independently and jointly. A recent survey described the quantities and distribution of Cu and Zn in this mesotrophic lake. The assessment included the North and South shore of the lake. The former receives water from the Ottawa River, while the latter is fed by the St. Lawrence River. Hazard Quotients (HQ) of 33.69 and 3.66 respectively for Cu and Zn were obtain during summer for fathead minnows on the North shore and 42.18 and 8.3 respectively on the South shore. HQs of 3.83 and 2.76 were obtained for Cu and

Zn respectively for bluegill sunfish on the North shore. The HQ values suggest that Cu and Zn levels in the water of Lake Saint-Louis produce undesired effects on spawning success for both species. Cu and Zn were tested in combination. Toxic units of 22.41 and 30.33 respectively were obtained for minnows from the North and South shore, while the value for sunfish was 3.96 suggesting a high susceptibility of eggs at spawning for both species.

An Ecological Risk Assessment of Lake Saint-Louis, Quebec: Risk of Methyl-Mercury in Yellow Perch, Pike and Walleye to Human Health. L.E. Tavera Mendoza[1], S.M. Ruby[1], P. Brousseau[2] and M. Fournier[2]. [1]Department of Biology, Concordia University, Montreal, QC; [2]INRS-Institut Armand-Frappier, Montreal, QC.

The risk of methyl-mercury to human health, following the consumption of Hg contaminated yellow perch, *Perca flavescens*, northern pike *Esox lucius*, and walleye, *Stizostedion vitreum* from Lake Saint-Louis, Quebec was evaluated. Hazard Quotients (HQ) were developed based upon the total body weight (kg) of males and females, the amount of fish consumed (g/d), the amount of methyl-mercury in fish muscle sampled from the North and the South side of Lake Saint-Louis during 1989 and 1992 and the reference dose for methyl-mercury as defined by EPA (1999). The HQ for sport fishers on the South shore who consumed an average of 40 g/d (280 g/week) was 1.3 and 1.1 for perch, 3.7 and 3.2 for pike and 3.3 and 2.8 for walleye for females and males respectively. These values suggest a high risk for the consumption of fish, with a greater risk to females relative to males. Similarly, HQ values of 0.65 and 0.56 for perch, 1.86 and 1.6 for pike and 1.6 and 1.4 for walleye were obtained for females and males respectively when calculations were carried out for the average Quebecer on the basis of consumption of 20 g/d (140 g/week) of fish. These data collectively suggest there is a health risk to humans from the consumption of fish from Lake Saint-Louis. Further monitoring of the fish from Lake Saint-Louis should be carried out.

PBDEs in Beluga Whales (*Delphinapterus leucas*) from the St. Lawrence Estuary. M. Lebeuf[1], K. Love[2] and S. Trottier[1]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [2]Southampton College, Long Island University, NY.

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in a wide range of manufactured products such as paints, polymers and textiles. Recent studies have shown that PBDEs are ubiquitous in the marine environment and bioaccumulate through the food web; however, the information on the levels of PBDEs in North American environments, particularly in the eastern part of Canada, remains very limited. In this study, we report levels of PBDEs in beluga whales from the St. Lawrence Estuary. Blubber samples were obtained from adult stranded beluga whales found dead on the shores of the St. Lawrence Estuary between 1997-1999. An analytical method was developed to determine levels of forty individual PBDEs in blubber samples using a gas chromatograph (GC) equipped with an ion trap mass detector operated in MS/MS mode. Preliminary data indicated that average concentrations of total PBDEs in female and male beluga whales were 432±189 and 300±135 µg/kg wet weight, respectively. This contrasts with levels of chlorinated contaminants (e.g., PCBs, DDTs) found in adult beluga whales which are generally much higher in males than in females. PBDE congeners were detected in only four of the seven homolog groups investigated and the distribution pattern of PBDE homolog groups was very similar between female and male beluga whales. Congener 2,2',4,4'-TeBDE (47) was clearly the most predominant brominated compound observed in all blubber samples, representing up to half of the total PBDE concentrations. Levels of 2,2',4,4'-TeBDE (47) are in the same range as many of the PCB congeners.

Levels of Toxaphene Congeners in a Marine Food Web from the St. Lawrence Estuary, Canada.

B. Gouteux[1], M. Lebeuf[2] and J.-P. Gagné[1]. [1]Institut des Sciences de la Mer, Rimouski, QC; [2]Ministère des Pêches et Océans, Institut Maurice-Lamontagne, Mont-Joli, QC.

Toxaphene is a major organochlorine contaminant present at elevated levels in many freshwater and marine environments in Canada. Reported concentrations in biota are generally similar to or higher than PCB or DDT compounds. However, there is still a lack of information on levels of toxaphene in the marine biota of the St. Lawrence system. In this study, samples of zooplankton, American eels, Atlantic tomcods, harbour seals and beluga whales from the St. Lawrence Estuary were analyzed. A new method using a gas chromatograph coupled to an ion trap detector operated in tandem mass spectrometry (MS/MS) was developed to quantify toxaphene congeners (CHBs) Parlar 26, 32, 40/41, 44, 50, 62 and 69 individually. Total toxaphene concentration (∑CHBs) was calculated as the sum of these specific congeners. Stable nitrogen isotope ratios ($\delta^{15}N$) were also calculated from the samples in order to determine the trophic positioning of the different organisms considered. All specific congeners, except CHB 69, were detected in the samples examined. CHBs 26 and 50 were the predominant congeners and they accounted for more than 75% of ∑CHBs. Preliminary results indicated that ∑CHBs (wet weight) increase with the trophic position. For instance, blubber of male belugas (mean $\delta^{15}N$=16.69%; mean ∑CHBs=2635 µg/kg) is clearly more contaminated than liver of Atlantic tomcods (mean $\delta^{15}N$=14.70%; mean ∑CHBs=68 µg/kg).

Sex-Reversal in *Xenopus laevis* Tadpoles Following Methanol and Atrazine Exposure During Late Metamorphosis. L.E. Tavera Mendoza[1], S.M. Ruby[1], P. Brousseau[2], D. Cyr[2] , M. Fournier[2] and D. Marcogliese[3]. [1]Department of Biology, Concordia University, Montreal, QC; [2]INRS-Institut Armand-Frappier, Montreal, QC; [3]Environment Canada, Montreal, QC.

Xenopus laevis tadpoles (Stage 56) were exposed to 21 µg/L of atrazine in $3.3x10^{-3}$ ml/L methanol carrier for 48 h during gonadal differentiation in late metamorphosis. Tadpoles were fixed for histological analysis and the gonads microdissected. Histological analysis of control, methanol carrier and atrazine exposed gonads were performed. There was an 80% incidence of sex- reversal among male testes in methanol exposed tadpoles. The ovotestis contained nests of primordial oogonia and primary oocytes. The later are not normally present in ovaries until Stage 66. Atrazine exposed tadpoles displayed agenesia in 40% of the testes but there was no evidence of sex-reversal. There was a 76 and 82% reduction of primary spermatogonia nests in carrier and atrazine- exposed testes respectively. The results suggest that while methanol causes sex-reversal in males, the presence of atrazine in the water, in combination with methanol blocks this effect.

Quantification of PAH Accumulation and Identification of Retene Metabolites in Rainbow Trout. R.S. Brown, S.P. Tabash, S.A. Hawkins and P.V. Hodson. Department of Chemistry and School of Environmental Studies, Queen's University, Kingston, ON.

The accumulation of waterborne polycyclic aromatic hydrocarbons (PAHs) in the yolk sac of rainbow trout sac fry varies significantly between PAHs. The bioaccumulation factor (BCF) is a function of K_{ow} and the rate of metabolism by MFO enzymes. To fully characterize PAH bioaccumulation, rates of uptake and metabolism must be determined. We have developed synchronous scanning fluorescence spectroscopy (SFS) methods to determine tissue PAH levels rapidly and with less cost than conventional methods. Body burdens in sac fry after waterborne PAH exposure were determined for retene, phenanthrene, benzo[a]pyrene, benzo[k]fluoranthene, and benzo[b]fluorene using SFS, with a typical recovery of 82±9%. The bioconcentration factors for retene and phenanthrene were calculated at <2 and 1,030, respectively. This is counter to K_{ow} predictions, and suggests that the rate of metabolism of retene is higher than Phenanthrene, supported by the increased MFO activity in fish

exposed to retene, and fluorescence measurements confirming retene metabolites. To identify the metabolites, methods for the separation of constituents in bile and liver homogenate were developed using high performance liquid chromatography (HPLC), with fluorescence and Mass Spectrometric (MS) detection. On-going studies involve using MS to confirm the presence of metabolites, and MS/MS to elucidate the structure of the metabolites.

Mining Environmental Effects Monitoring

Eskay Creek Mine Environmental Effects Monitoring (EEM) Program. F. Landry[1], F.M. Murphy[2], I.D. Sharpe[3], A. Fikart[4] and P.M. Chapman[1]. [1]EVS Environment Consultants, North Vancouver, BC; [2]Homestake Canada Inc., Smithers, BC; [3]Ministry of Environment, Lands and Parks, Smithers, BC; [4]Resource Management and Environmental Studies, University of British Columbia, Vancouver, BC.

The Eskay Creek gold/silver mine is located in northwestern British Columbia (BC). In 1997, an EEM program was designed and implemented by EVS Environment Consultants, in conjunction with Homestake Canada and the Ministry of Environment Lands and Parks. The objectives of the program are to assess whether there are exceedances of the BC water quality guidelines in the receiving environment and whether resident aquatic fauna and flora, such as benthic invertebrates and periphyton are adversely affected. Over the past four years, several study components have been included in the program: annual water quality monitoring; quarterly toxicity testing of discharges and reference sites; sediment collection for chemistry characterization and toxicity testing; benthic invertebrate collection; periphyton collection; and bivalve bioaccumulation studies. A total of 14 sites including reference, initial dilution zone, near-field, and far-field stations have been monitored in the receiving environment. Impacts to local aquatic biota appear to be primarily restricted to the outflow from one discharge station. Some metal concentrations in the discharge water and sediments at this station were above criteria and appear to be causing effects on benthic and periphyton communities. No consistent adverse impacts were observed on the aquatic communities at any other stations in the receiving environment.

Differentiating Chemical and Physical Risks from a Mine Tailings Release in Idaho. J. Fisher[1,2], R. Gilmore[1] and J. Houghton[1]. [1]Pentec Environmental, Edmonds, WA; [2]ENTRIX, Inc., Seattle, WA.

Chemical and physical risks to redband rainbow trout (*Oncorhynchus mykiss*) were assessed in a small stream that received arsenic-enriched tailings following the breach of a retention dam. Water, sediment, fish health and abundance, macroinvertebrates, and physical habitat elements were sampled to determine how biota were exposed and/or affected. Background metal concentrations in water and sediment generally did not differ significantly from those measured within the tailings depositional area, although concentrations of some metals in sediment within the depositional area exceeded some effects thresholds. The redband trout population within the depositional area (0.1 trout/sq. m) was significantly reduced relative to that upstream of this area (0.25/sq. m). Macroinvertebrates more tolerant of physical and chemical disturbance (e.g., Chironomidae) were found within the depositional area but there was no loss of species found in background stations upstream. Some physical habitat disturbance identified was related to the initial tailings release and subsequent removal activities, but the predominant impact to habitat was from historic land use practices (e.g., channelization). As the chemical risks were found to be insignificant at this site, the restoration of the trout population in the long term is dependent upon restoration of properly functioning riparian conditions. The importation of large wood and restoration of sinuosity could rectify habitat deficits to support a greater trout population in the near term.

Toxicity of Four Pure Metals to *Lemna minor* using Environment Canada Procedure EPS 1/RM/37 - March 1999. M. Moody. Saskatchewan Research Council, Saskatoon, SK.

The objective of this study was to assess the toxicity of four metals potentially present in liquid wastewaters from metal mining. The method used was the Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte *Lemna minor* (EPS 1/RM/37 - March 1999). While it is recognized that metal mining wastewaters are complex mixtures, this work indicates the sensitivity of *L. minor* to single metals when tested in an inorganic medium free of chelators. Two endpoints were used in determination of IC_{25} for Cd, Cu, Ni and Zn. The increase in the number of fronds and mean dry weight (as an indication of growth) were measured at seven days. Two 3-frond plants were grown in 150 ml modified APHA medium in a series of dilutions of each toxicant under 24 h light at 25°C. Based on frond count the order of decreasing toxicity of the metals was Ni=Cd=Cu>Zn. Dry weight was a less sensitive endpoint for Cd, Cu and Zn.

Fine Grained Particulates – Assessment of Aquatic Effects. G.R. Craig. G.R. Craig & Associates Inc., Bolton, ON.

Water quality criteria limit increases in suspended particulate (TSS) relative to background levels or changes in water clarity and light transmission. While permanent change in water quality due to suspended particulate can change habitat and use, the presence or absence of effect resulting from transient exposures up to several months is dependent on many factors. The physical and chemical nature of particulates is essential to determining the potential for concentrations related effects. Historical studies often fail to consider important factors that determine the magnitude of effects but these studies are imbedded in current criteria. Factors such as chemical associated with coal dust and pulp fibre, density and settling rates of different grains (sand, silt, clay) angular or smooth surfaces that govern abrasive qualities, size related to organism gill structure and fundamental composition that determines plasticity, all complicate the simple concentration dependent paradigm that is often used in exposure assessments. The above confounding factor will be illustrated to assist evaluator in making more realistic impact assessments on fish communities.

Soil and Sediment Toxicity Testing

St. Clair River Sediment Toxicity Identification Evaluation. T. Moran[1], T. Kierstead[1], S. Munro[2] and P. Child[3]. [1]Pollutech Enviroquatics Ltd., Point Edward, ON; [2]Sarnia-Lambton Environmental Association, Sarnia, ON; [3]Investigative Science Inc., Burlington, ON.

In the last decade, substantial improvements have been made to the water quality of the St. Clair River. Despite the improvements, impairments to beneficial uses have been identified by the IJC's St. Clair River RAP. Efforts are focused on finding solutions to the remaining problems. Three areas of sediment, located along the Canadian side of the St. Clair River, have been classified as "Priority 1" zones as a result of poor sediment quality. Using an integrated sediment triad approach, the LIS work found that at seven station locations, within these three sediment zones, there was "strong evidence of pollution induced degradation from sediments." Part of the rationale for this conclusion was that acute and/or sublethal/chronic toxicity to either fathead minnows and/or to benthic organisms was observed. Further laboratory work found a number of contaminants to be potentially associated with acute toxicity to fathead minnows. To help understand what in the sediment may be causing a problem, more formal toxicity identification evaluation (TIE) procedures were adopted. Since toxicity is more likely associated with contaminants found in the sediment porewater, the TIE procedures were applied to porewater extracted from the aquatic sediments. The modified U.S. EPA Phase I TIE

procedures were used to help categorize the type of toxicant. Surprizingly, the likely source of toxicity was not found to be associated with the typical sediment contaminants for the area (PAH's, chlorinated hydrocarbons, etc.), but was found to be associated with an organic amine compound.

Levels and Source Apportionment of Polycyclic Aromatic Hydrocarbons (PAHs) and Sulfur Heterocycles (PASHs) in Sediments and Mussels. J. Hellou[1], T. King[1], J. Leonard[1], T.G. Milligan[1], S. Stellar[1], P. Yeats[1] and V. Zitko[2]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Nova Scotia; P.O. Box 1006, Dartmouth, NS; [2]Department of Fisheries and Oceans, Biological Station, St. Andrews, NB.

The title compounds, including both the parents and their alkylated derivatives, were determined in surficial sediments and blue mussels (*Mytilus edulis*), collected at 19 and 16 sites, respectively, along the shores of Halifax Harbour, Nova Scotia, Canada. The objective was to determine the relationship between the profiles observed in sediments and in mussels and to explore the possibility of deducing the patterns of their sources, deposition, and circulation in the harbour. Combustion-derived compounds predominated in both sets of samples. More variability was observed in the level of petroleum-derived PAHs in mussel than sediment samples. Alkylated derivatives represented between 7-50% of the sum of target compounds in mussels and 15-30% of the sum in sediments. Three sites displayed the highest levels of PAHs and PASHs, organic carbon (OC) and sulfur (S) in sediments. One of these sites also had high levels of PAHs and PASHs in mussels. Biota sediment accumulation factors (BSAF, dry/dry weight) varied throughout the harbour, ranging from means of 0.006-1.26. In general, fluoranthene had the highest BSAF. Mussels and sediment results are compared to published data.

Organic Contaminants, Including PAHs, PCBs and DDTs in Sediments Collected Under Aquaculture Cages. J. Hellou[1], K. Haya[2], L.E. Burridge[2], S. Steller[1] and C. Chou[1]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [2]Department of Fisheries and Oceans, Biological Station, St. Andrew's, NB.

During the summer of 1998, small and large food pellets, fish oil, used as an ingredient to prepare feed, as well as 15 sediments collected around salmon aquaculture sites were analysed. In New Brunswick, the salmon aquaculture sites are concentrated in the south western Bay of Fundy. Since the aquaculture industry may have an input of anthropogenic chemical wastes at these sites, the level of priority organic contaminants was examined under the cages. Samples were analysed for 159 PCB congeners, 31 parental and alkylated polycyclic aromatic hydrocarbons (PAHs) and 12 organochlorine pesticides. These chemicals would represent compounds that are unintentionally used by the industry. Combustion derived PAHs were most abundant in sediments, with alkylated PAHs representing between 3-27% of the sum of PAHs. Naphthalenes were detected in sediments, food pellets and fish oil, while alkylated phenanthrenes were only detected in sediments. Trace levels of resolved or co-eluting PCB congeners were detected in some of the sediments (<4 µg/kg, dry), with IUPAC congener numbers 153/132/168 and 138/160/163/158 being most abundant. As expected, the DDTs predominated within the pesticides series. Concentrations of organochlorine compounds tended to be higher where organic carbon content was more elevated, but PAHs did not follow this trend.

Application of the BEAST Biological Sediment Guidelines to Hg Contaminated Sediment in Peninsula Harbour, Lake Superior. D. Milani, S.P. Thompson, T.B. Reynoldson and L.C. Grapentine. Environment Canada, National Water Research Institute, Burlington, ON.

Peninsula Harbour, located on the northeastern shore of Lake Superior has been identified as an Area

of Concern due to elevated levels of Hg. Nine sites (ranging from 0.35-6.97 mg/kg Hg) were sampled, and the sediment was assessed using the BEAST biological sediment guidelines (analysis of benthic community structure, environmental parameters, and functional response in sediment toxicity tests with benthic invertebrates: *Hyallea azteca*, *Chironomus riparius*, *Hexagenia* spp. and *Tubifex tubifex*). Data were analysed using ordination and the relationship between the environmental variables and the biological data was examined by correlation analysis of the environmental variables with ordination vector scores. The benthic community and functional response were categorized by the guidelines to four levels of toxicity or community stress (none, potential, stressed or toxic, or severe) for each site. *H. azteca* survival and growth demonstrated the greatest response; severe toxicity was evident at two sites, and medium toxicity at two other sites. Of these four sites showing toxicity, two showed degradation of invertebrate communities. These two sites were also above the provincial sediment quality guidelines LEL, and one was above the SEL. Hg concentration showed a significant ($p \leq 0.05$) relationship with the ordination axes for both the toxicity test response and for the community structure. Further work, in particular bioaccumulation studies, would help explain the role of Hg in toxicity in Peninsula Harbour.

Biological Impacts of Metals in Lake Sediments Near Sudbury, Ontario. U. Borgmann, W.P. Norwood, T.B. Reynoldson and F. Rosa. Environment Canada, National Water Research Institute, Burlington, ON.

Metal contamination of lake sediments from the Sudbury area is well documented, but there has been no detailed examination of its biological impact. Most biological studies in the Sudbury area have focused on the impact of sulfur emissions and lake acidification, particularly effects on plankton and fish. Metal-induced impacts, if present, could be masked by, or indistinguishable from, acidification effects. Since metals bind strongly to sedimenting particles, the highest concentrations of metals are found in the deep sedimentary basins of lakes, and benthic organisms are likely to be among the most strongly affected. A comparison of 12 circum-neutral (i.e., non or mildly acid-impacted) lakes at 6-154 km from the smelter at, Copper Cliff, Sudbury, revealed increased metal contamination in deep (≥10 m) sediments, decreased abundance of some benthic invertebrates, and increased sediment toxicity to several species. Increased concentrations of Cd, Co and Ni in sediments were associated with increased bioaccumulation in *Hyalella* in the laboratory, but only Ni bioaccumulated in sufficient amounts to be toxic. Ni concentrations in the sediment are sufficient to cause 25% mortality in chronic tests with sediments collected up to 20 km, on average, from Copper Cliff.

The Effects of Naturally Occurring PAH Compounds on the Early Life Stages of Fathead Minnows. M.V. Colavecchia[1], P.V. Hodson[1] and J.L. Parrott[2]. [1]Department of Biology, Queen's University, Kingston, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

This study examines the effects of exposure to naturally occurring PAHs on the early life stages (ELS) of fathead minnows (*Pimephales promelas*). Sediments were collected from sites along tributaries of the Athabasca River (AB), which are within and outside natural oil sand deposits. ELS toxicity test (static daily renewal) was performed using site sediments, bitumen and controls. Ten fertilized eggs were placed in glass containers with varying amounts of sediments (25, 12.5 and 6.25 g), 800 ml of dechlorinated water and moderate aeration. Sediments and water were allowed to settle for 12 h. Eggs and larvae were checked daily for mortality, hatching and deformities. At the end of 12 d, total body length, wet and dry weights were measured. Preliminary experiments show significant egg and larval mortality with embryos exposed to natural oil sands bitumen. All larvae exposed to bitumen sediments died. Larval deformities were significantly higher in larvae exposed to bitumen compared to controls and reference sediments. Deformities included subcutaneous yolk sac and heart edema, hemorrhages, craniofacial malformations and curved spines. Exposure to reference sediments and

controls showed negligible embryo mortality, excellent larval survival and no deformities. ELS sediment toxicity tests may prove to be valuable and predictive tools in the monitoring and assessment of petroleum pollution.

Depuration of Trace Metals in Laboratory Exposed *Tubifex tubifex* and Corresponding Metallothionein Concentrations. P.L. Gillis[1,2], D.G. Dixon[1] and T.B. Reynoldson[2]. [1]Department of Biology, University of Waterloo, Waterloo, ON; [2]Environment Canada, National Water Research Institute, Burlington, ON.

Laboratory cultured *Tubifex tubifex* were exposed to sediment artificially contaminated with Pb, Cd and Ni for a period of two weeks. The worms significantly ($p < 0.001$) accumulated all three metals, resulting in a 10-1,200 fold increase in tissue residue. Bioaccumulation factors were 7.2 for Pb, 22.7 for Cd and 3.5 for Ni. Metallothionein concentration also increased significantly ($p < 0.001$) compared to pre-exposed worms. Following exposure the worms were transferred to either clean sediment (up to 10 weeks) or clean water (up to 24 h) to determine any changes in tissue residues and metallothionein concentrations. The concentration of Pb, Cd and Ni in the worm tissue declined with increasing time in clean sediment. By the end of the sediment depuration period the worms lost 67-90% of the accumulated metal but the levels were still 2-415 fold higher than the pre-exposed condition, whereas the corresponding metallothionein concentration had returned to within 13% of the pre-exposed level. In animals that were transferred to clean water to depurate none of the variables had significantly ($p < 0.05$) declined after 24 h. Earlier experiments found that a period of 24 h in water was adequate time for *T. tubifex* to clear its gut contents, thus suggesting that Pb, Ni and Cd are accumulated in the tissues of the worm.

Maintaining Water Quality in Static Sediment Toxicity Tests. U. Borgmann and W.P. Norwood. Environment Canada, National Water Research Institute, Burlington, ON.

Overlying water quality often deteriorates rapidly in standard sediment toxicity tests. One solution is continuous water renewal, but this is labour intensive or requires elaborate and expensive automated equipment. Excellent overlying water quality can, however, be maintained under static conditions if a large water to sediment ratio is used. Non-standard containers, such as Imhoff settling cones, provide adequate sediment depth with as little as 10-15 ml of sediment and 1 L of overlying water. In addition to negating the need for water renewal, static tests in cones provide large water volumes for chemical analysis at the end of the test. All contaminants diffusing into overlying water are retained during the test and metal concentrations measured in overlying water correlate much better with bioavailability than in beaker tests. Sorting and counting animals is also easier because smaller sediment volumes are used.

Hyalella Need Bromide. U. Borgmann and W.P. Norwood. Environment Canada, National Water Research Institute, Burlington, ON.

Artificial media are essential for several applications in aquatic toxicology. They allow toxicity tests to be conducted under standardized conditions with identical water quality worldwide, and permit detailed investigation of the effect of individual dissolved chemical components on contaminant toxicity. *Hyalella azteca* is one of the most widely used invertebrates for sediment toxicity testing in North America, and is also used in South America and Europe. Success in the use of artificial media for testing with *Hyalella* has often been inconsistent, at least in part because many researchers are unaware that *Hyalella* requires bromide. Na, bicarbonate, Ca and bromide must be present together in solution to optimize long term (4-10 week) survival. Ca is not beneficial to *Hyalella* in the absence

of bromide, and $CaCl_2$ is toxic if bromide concentrations are low. No other ions are needed for survival, but Mg and K are needed for optimum growth and reproduction.

Endocrine Disruptors

A Canadian Perspective on Endocrine Disrupting Substances in the Environment.

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Although reproduction and development are major endpoints for environmental and human health assessments in many federal government programs, growing concern about risks posed by endocrine disrupting substances (EDS) has highlighted the need to detect subtle effects mediated through the endocrine system. Internationally, screening and testing programs in the United States, Japan and Europe, and the activities of international agencies such as the OECD will have a profound influence on how Canada will address this issue in the future. It is critical that Canada be proactive, identify knowledge gaps from a Canadian perspective, and anticipate international developments that may influence Canadian policy. The complexity and urgency of the EDS question spurred the Five Natural Resource Departments Endocrine Disrupting Substances (5-NR EDS) Working Group to hold a multi-departmental, multi-stakeholder, workshop to address key issues in assessing risks to Canadians and the Canadian environment. The following is a summary of the major conclusions, research priorities and recommendations from the workshop held at the Grandview Inn, in Huntsville, Ontario, Feb. 13-17, 2000.

Vitellogenin as a Biomarker of Exposure to Environmental Estrogens in PEI Streams. J.P. Sherry[1], R. Mroz[2], W.R. Ernst[2], M. Servos[1], C. Tinson[1], M.E. McMaster[1] and R. Halford[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Environment Canada, Atlantic Region, Dartmouth, NS.

Many Prince Edward Island (PEI) streams flow through heavily cultivated watersheds. The potato is farmed intensively through much of the island. Potato crops are typically given multiple applications of pesticides through the growing season. Some of the applied pesticides are suspected endocrine disrupters. In the summer of 1999, we caged juvenile rainbow trout in PEI streams to test whether exposure to ambient water in streams close to potato farms could trigger an estrogenic response in fish. Induced vitellogenin (Vg), a biomarker of exposure to estrogenic substances, was measured by enzyme-linked-immunosorbent-assay (ELISA). Some fish plasmas misbehaved in the ELISA, possibly because of degradation of plasma proteins. We will describe a modification of the ELISA protocol that facilitates the analysis of such samples. Caged fish were also injected with 17β-estradiol to test for possible anti-estrogenic effects and to confirm that the fish were capable of responding to an estrogen in this particular experiment. The time course of the Vg response to a single i.p. injection of 17β-estradiol was established in a laboratory experiment.

Characterization and Assessment of a Bioassay for the Detection of Responses to Environmental Estrogens in Trout. A. Gamble[1], P.V. Hodson[2], K. Solomon[3], P.-D. Hansen[4], B. Hock[5], A. Marx[5] and J.P. Sherry[6]. [1]Department of Environmental Biology, University of Guelph, Guelph, ON; [2]Department of Biology, Queen's University, Kingston, ON; [3]Centre for Toxicology and

Department of Environmental Biology, University of Guelph, Guelph, ON; [4]Department of Ecotoxicology, Technical University of Berlin, Berlin, Germany; [5]School of Botany, Technical University of Munich, Freising, Germany; [6]Environment Canada, National Water Research Instate, Burlington, ON.

A laboratory based bioassay was developed for the detection and measurement of estrogenic responses in rainbow trout (*Oncorhynchus mykiss*). Induction of plasma vitellogenin (Vg), which was measured by enzyme-linked-immunosorbent-assay (ELISA), was the response parameter. Induction of Vg in juvenile fish is now widely accepted as an appropriate biomarker for exposure to estrogenic substances. A brief description of the ELISA's performance characteristics will be provided. The performance of the static renewal bioassay was characterized by exposure of fish to solutions of 17β-estradiol. In a series of experiments we assessed the influence of renewal ratio and renewal rate on the ability of the bioassay to detect low concentrations of 17β-estradiol. The bioassay was used to screen effluents from Ontario pulp mills and a petroleum refinery for possible estrogenicity. Trout were exposed to pulp mill effluent that was spiked with 17β-estradiol to test whether their response, expressed as Vg induction, to a known estrogen was modulated under those conditions.

Detection and Characterization of Sex Hormone Ligands Associated with Pulp Mill Effluents in Canada. M.L. Hewitt[1], J.P. Sherry[1], M.E. McMaster[1] and G.J. Van Der Kraak[2]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Zoology, University of Guelph, Guelph, ON.

The effects of treated pulp and paper mill effluents on the reproductive fitness of wild fish has been well documented in Canada. Industry-wide process modifications designed to reduce acute toxicity, BOD and organochlorine discharges have resulted in improved reproductive capacity in fish at many sites, however, responses have persisted in fish exposed to effluent at several mills. Presently, the chemicals responsible for these effects are not known. Recent development of *in vitro* assays for chemicals with the ability to function as sex hormone ligands now provides the opportunity evaluate their possible role in persistent responses. Effluents from 10 mills across Canada were collected before and after treatment, lyophilized, extracted and tested for the potential to: [1] compete for goldfish (*Carassius auratus*) sex steroid binding protein (SSBP); [2] compete for hepatic estrogen receptors (ER) in rainbow trout (*Oncorhynchus mykiss*); and [3] induce vitellogenin production in primary cultures of rainbow trout hepatocytes. Similar trends in hormonal activities were detected in the effluents studied and activities were generally reduced after secondary treatment. Application of these assays has also shown that fish rapidly accumulate sex steroid ligands from treated effluents and eliminate them after a short holding period in clean water. Discrimination between activity derived from endogenous hormone and chemicals accumulated from effluent has been made.

Effects of the Synthetic Estrogen 17α-Ethynylestradiol on Lower-Trophic-Level Organisms. K. Kidd[1], D. Findlay[1], M. Paterson[1], C. Podemski[1], A. Salki[1], L. Vandenbyllaardt[1] and K. Liber[2]. [1]Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, MB; [2]University of Saskatchewan, Saskatoon, SK.

Little is currently known about the effects of hormone mimics on the abundances and species composition of lower-food-web organisms. We conducted an enclosure study to examine the effects of a potent estrogen mimic, 17α-ethynylestradiol (EE2), on the algal, microbial, zooplankton and benthic invertebrate communities in an oligotrophic lake at the Experimental Lakes Area, northwestern Ontario. Over a 6 week period we added EE2 continuously to 6 treatment enclosures (2 m dia. by 2 m deep) using addition rates determined from a previous enclosure study of this hormone. Weekly water column samples and plankton tows were collected for EE2, algal, zooplankton, and microbial

community analyses, and bi-weekly for nutrient analyses from the treatment and control enclosures. Benthic invertebrate communities were examined using grabs collected at the beginning and end of the experiment. Mean water column concentrations in the treatment enclosures ranged from 4-559 ng EE2/L over the six weeks. The phytoplankton biomass was dominated by chrysophytes; no concentration-dependent effects of EE2 were observed in either the community composition or in the biomass of algae. Bacterial biomass (0.1-0.2 mg/L) and dissolved nutrient concentrations were similar in all enclosures throughout the experiment. Dominance in the zooplankton community shifted towards cyclopoid zooplankton in weeks 2-6, but no differences were observed between the treatment and control enclosures. No significant effects of EE2 were observed on all benthic invertebrates examined. In conclusion, the results indicate that EE2 does not impact abundances and composition of lower-trophic-level organisms at the concentrations tested (<560 ng/L).

Isolation and Identification of EROD/Vitellogenin Inducers from Several Pulp Wood Species. M. Kohli[1], J.L. Parrott[1], J.P. Sherry[1], M. Comba[1], B. Schrag[2] and J. Carey[1]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Chemical Ecotoxicology, Institute of Umweltforshungszentrum, Permoserstr, Leipzig, Germany.

Over the last decade, our research team has been involved in the characterization of the chemical composition of the pulp mill effluents and their biological impact to the environment. One particular focus has been on the identity and origin of compounds responsible for several physiological responses in fish. We have previously suggested that the compounds responsible for MFO induction in fish exposed to these effluents were members of the stilbene family that were naturally present in some wood species. In the current study the stilbene content of several pulp wood species (*Pinus sylvestris, Abies balsamea, Picea mariana, Thuja occidentalis, Tsuga canadensis* and *Populus tremuloides*) collected from eastern and western Canada were investigated. Three stilbenes were isolated in crystalline form from *P. sylvestris* and their chemical structures confirmed. All these compounds and their chlorinated products demonstrated a positive MFO response in liver cell lines. However, the values were higher in case of chlorinated products. Some of the wood extracts also induced Vg in rainbow trout hepatocytes.

Masculinisation of Adult Female Mosquitofish (*Gambusia affinis affinis*) Exposed to Pulp and Paper Mill Wastewaters. R.J. Ellis[1,3], M.R. van den Heuvel[1], T.R. Stuthridge[1], L.H. McCarthy[2] and D.R. Dietrich[4]. [1]NZ Forest Research Institute Ltd., Rotorua, New Zealand; [2]Ryerson Polytechnic University, Toronto, ON; [3]University of Waikato, Hamilton, New Zealand; [4]University of Konstanz, Konstanz, Germany.

International studies have noted effects of pulp and paper effluents on the reproductive physiology of fishes including reduced plasma sex steroid levels, reduced fertilisation/hatching success, delayed maturity and modification of secondary sexual characteristics. The present study is part of an ongoing programme to determine the potential impacts of a New Zealand pulp and paper mill's effluent on fish populations. Adult female mosquitofish, *Gambusia affinis affinis*, were exposed to pulp mill wastewater prior to and post secondary treatment in an aerated stabilisation basin system for 21 d. Occurrence of gonopodial development (masculinisation) was noted. The use of filtered (GFC) and unfiltered samples initiated examination of the route of exposure. The incidence of gonopodial development was noted in all the unfiltered samples at ecologically relevant concentrations (15% by volume), with significantly less in the post aeration treatment effluent. Filtered samples revealed significantly less incidence of gonopodial development compared to unfiltered. Male mating behaviour was observed in masculinised adult female mosquitofish. Thus, the above data suggests significant endocrine modulating and potential population level effects of combined TMP/BKM effluent in this species.

Effects of Toxaphene on Hepatocytes of Yellowtail Flounder (*Pleuronectes ferrugineus*): An Ultrastructural and Histochemical Study. C.M. Murphy and G.E. Fåhræus-Van Ree. Department of Biology, Memorial University of Newfoundland, St. John's, NF.

The ultrastructural and histochemical effects of dietary Toxaphene were investigated on livers of laboratory-bred, sexually-immature female and male yellowtail flounder (*Pleuronectes ferrugineus*). The fish were fed twice daily for two weeks with uncontaminated food or food contaminated with hexane (the solvent of Toxaphene), or three different concentrations of the pesticide (0.002, 0.02 and 0.2 mg/kg fish/day). Ultrastructural examination of the lipids stained with ρ-phenylenediamine showed a significant increase in the size of lipid droplets in the hepatocytes of females exposed to the highest concentration of Toxaphene (0.2 mg/kg fish/day), suggesting a sex-specific and dose-dependent Toxaphene response. In addition, Toxaphene exposure resulted in an increase of lipolytic activity, an overall reduction and increase in fenestrations of rough endoplasmic reticulum (RER), and proliferation of smooth endoplasmic reticulum (SER) within the hepatocytes of both sexes, indicating an impairment of lipid and protein metabolism. Preliminary results revealed that lysosomal trimetaphosphatase activity was increased in Toxaphene-exposed animals, suggesting an increase in the number and/or size of lysosomes. The storage of endogenous pigments (hemosiderin and lipofuscin), determined by computerized image analysis, were also affected by Toxaphene. The amount of hemosiderin, determined with Turnbull blue stain, was significantly lower in the liver tissue of both sexes exposed to the second highest concentration of Toxaphene (0.02 mg/kg fish/day) relative to the other treatment groups, suggesting an impaired turnover of erythrocytes. The males showed a dose-dependent increase of the autofluorescence of lipofuscin within the hepatocytes of Toxaphene-exposed animals, indicating an increased degradation of cellular components. It is concluded that Toxaphene, an endocrine disrupter, is potentially toxic to yellowtail flounder.

Pulp and Paper Environmental Effects Monitoring

Pulp and Paper Environmental Effects Monitoring (EEM) in the Atlantic Region: A Comparison of Cycle 1 and 2 Sublethal Toxicity Results. K.K.J. Kim[1], J.K. Parker[1], J.J. Silver[1] and W.R. Parker[2]. [1]Environment Canada, Environmental Protection Branch, Dartmouth, NS; [2]Environment Canada, Environmental Protection Branch, Fredericton, NB.

The *Pulp and Paper Effluent Regulations* under the *Fisheries Act* require all mills to limit the biochemical oxygen demand (BOD), total suspended solids (TSS), and the acute lethality to fish of the effluent. As well, all mills must conduct an environmental effects monitoring (EEM) program on a regular basis. One required component of the EEM program is semi-annual sublethal toxicity tests to measure the survival and growth of early life stage development of fish, invertebrate reproduction and alga toxicity on samples of mill effluent. A review of Cycle 1 and Cycle 2 sublethal toxicity test data indicates that the quality of mill effluent has improved for most of the mills in the Atlantic Region from Cycle 1. In spite of the improvement in effluent quality, the pulp and paper mill effluents still elicit toxic responses from invertebrates and algae. Trends in sublethal toxicity related to BOD, TSS, different mill processes, effluent treatment processes, type of wood furnish used, and the type of product produced are examined.

Use of Benthic Communities as a Tool to Assess Impacts of Québec, Pulp and Paper Mill Effluents on the Freshwater Receiving Environment. R. Chabot and C. Langlois. Environnement Canada, Direction de la protection de l'environnement, Montréal, QC.

As part of the Second Cycle of the Environmental Effects Monitoring (EEM) Program conducted under

the *Canadian Pulp and Paper Effluent Regulation*, benthic invertebrate community surveys (BICS) were conducted in 1998-1999 by 37 Québec pulp and paper mills discharging their effluent in freshwater. The objective of this communication is to show the usefulness of BICS as a tool to assess spatial and temporal changes of the effects of mill effluents on the receiving environment. Using a control-impact design, the stations of the exposure zones (near-field and far-field) were located within the effluent mixing zone, delineated by the 1% effluent concentration. Most studies showed comparable habitats between reference and exposure zones, thus reducing many confounding factors observed during Cycle 1. Ponar grab was the most commonly used sampling device, although some mills used the Hess sampler or Hester-Dendy artificial substrates. The samples were sieved with a mesh size of 500 µm and the invertebrates were generally identified to the genus or the species level. For many mills, results of the BICS performed during Cycle 2 showed an improvement from those of Cycle 1. Comparison of results between both cycles also contributed to discriminate mill-related impacts from other local pollution sources to aquatic environment. Severe organic enrichment of the receiving water was less frequent than during Cycle 1. Implementation of secondary treatment in 1995 contributed to major improvement of the habitat quality of many receiving waters. The benthic community in the exposure area of some mills went from severely disturbed (Cycle 1) to levels comparable to those of the reference zone (Cycle 2). Thus the Cycle 2 results suggest that BICS is an effective tool to assess the spatial and temporal changes of the effluent impact on the receiving freshwater environment.

Environmental Effects Monitoring at Ontario Pulp and Paper Mills: Effects of Mill Effluent on Invertebrates. A.I. Borgmann, K. Tuininga and H. Sun. Environment Canada, Environmental Protection Branch, Downsview, ON.

The primary objective of the Environmental Effects Monitoring (EEM) program is to assess the adequacy of national regulations for protecting fish, fish habitat and the use of fisheries resources, by assessing the magnitude and the spatial extent of site-related effects and temporal changes through successive cycle of EEM. Benthic invertebrate community surveys were conducted as one component of the first two cycles of EEM, a requirement under the *Canadian Pulp and Paper Effluent Regulations*. Following implementation of secondary treatment, significant improvement of benthic communities was observed at most sites in Ontario. Utilizing the abundance data of pollution sensitive (*Ephemeroptera*) and pollution tolerant (*Chironomid*) species, evidence has emerged that the Benthic Invertebrates Surveys effectively demonstrate magnitude and spatial extent of mill effluent related effects.

A Comparison of Fish Survey Results from Two Consecutive Environment Effects Monitoring Cycles at Ontario Pulp and Paper Mills. K. Tuininga and A.I. Borgmann. Environment Canada, Environmental Protection Branch, Downsview, ON.

The primary objective of the Environmental Effects Monitoring (EEM) program is to assess the adequacy of national regulations for protecting fish, fish habitat and the use of fisheries resources, by assessing the magnitude and the spatial extent of site-related effects and temporal changes through successive cycles of EEM. Fish surveys were conducted as one component of the first two cycles of Environmental Effects Monitoring, a requirement of the *Pulp and Paper Effluent Regulations*. Following implementation of secondary treatment, benthic communities showed significant improvement at most sites while fish results were more variable. Cycle 2 fish survey results are presented and compared to Cycle 1 results where possible.

Using Bivalves in British Columbia Pulp and Paper EEM Program Fish Surveys. M.E. Hagen and J.M. Boyd. Environment Canada, Environmental Protection Branch, North Vancouver, BC.

Pulp and paper mills discharging to the aquatic environment are required by the *Pulp and Paper Effluent Regulations* to conduct an Environmental Effects Monitoring (EEM) program. In Cycle 1 and Cycle 2, marine-discharging mills in British Columbia used a variety of bivalve methods to satisfy the fish survey component of their EEM program. Methods included collection of wild bivalves (blue mussels [*Mytilus edulis*], and Pacific oyster [*Crassostrea gigas*]), caged bivalves using *M. edulis*, and an on-shore bioassay using *M. edulis*. Methods are compared and contrasted, and the effectiveness of the methods to achieve EEM objectives is discussed.

Benthic Invertebrates for Upper Fraser River EEM Cycle 2: Hess vs. Reference Condition Approach Methods and Results. L.U. Young[1], T.B. Reynoldson[2], E. Dobson[1] and L.W. Dwernychuk[1]. [1]Hatfield Consultants Ltd., West Vancouver, BC; [2]Environment Canada, National Water Research Institute, Burlington, ON.

ABSTRACT

In the fall of 1998, benthic invertebrate samples were collected in the vicinity of Prince George and Quesnel for Cycle 2 EEM monitoring of four pulpmill effluent discharges. Two different assessment approaches were used: first, standard EEM protocols were followed using a Hess sampler at 24 stations, samples were sieved at 500 µm; second, one 3 min kick sample of invertebrates was collected at the same stations (except four due to slope) and sieved at 400 µm using Reference Condition Approach (RCA) protocols.

Results of the Hess method indicated greater abundance of invertebrates in the Prince George region relative to Quesnel. Number of taxa increased from reference to far-field areas in the Prince George region; numbers of taxa were consistent throughout the Quesnel region. No significant statistical differences (using ANOVA) were observed in abundance and number of taxa among areas for the entire upper Fraser River Cycle 2 program. Therefore, no toxic or enrichment effect was observed that could be related to pulpmill effluent in any exposure area using Hess sampling.

Reference Condition Approach invertebrate data indicated similar trends in abundance and number of taxa as Hess samples. Results of a completed RCA analysis are compared to the statistical analysis and weight-of-evidence approach used for data generated from Hess samples.

INTRODUCTION

The EEM Technical Guidance Document (Environment Canada 1998) for pulp and paper mills recommends a Control/Impact design for freshwater rivers, and the use of Neill-Hess cylindrical samplers for erosional habitats. In areas where the Reference Condition Approach (RCA) (Reynoldson *et al.* 1997) has been developed, the use of RCA methods and analyses should also considered for the EEM invertebrate community survey. The Cycle 2 invertebrate survey was designed as a control/impact survey in Prince George and Quesnel regions, with Hess sampling used as the mill-based program and RCA conducted with the support of Environment Canada. These two methods/approaches are compared herein. Methods, results and conclusions also have been reported in Hatfield Consultants Ltd. (2000) and T. Reynoldson (unpubl. data).

MATERIALS AND METHODS

Basic design for two regions with two discharges each: erosional habitat, total of 24 stations; 3 reference stations per region; 3 near-field stations downstream of each discharge; and 3 far-field stations per region.

Hess Method (September 1998): 0.1 m^2 sampler with 200 µm mesh, at 25-30 cm depth; three subsamples per station, not pooled; sieved in laboratory at 500 µm; identified to lowest reasonable taxonomic level (usually to genus), these taxa used for statistical analyses; and water samples, visual observations of sediment structure.

RCA Method (October 1998): 3 min kick sample, one per station, zig-zag pattern following the shoreline, at approximately 80 cm depth; triangular kick net with 400 µm mesh; 4 stations inaccessible for RCA (too steep); sieved in laboratory at 400 µm; identified to lowest taxonomic level; family level used for statistical analyses; and several habitat variables collected per RCA, including substrate matrix rating.

RESULTS AND DISCUSSION

Densities and number of taxa:

Generally, benthic invertebrate abundance was higher in Prince George relative to Quesnel; considerable variability was observed in each area (applies to both Hess and RCA samples). Density from Hess samples cannot be compared to 3 min kick samples for quantitative analyses. Number of taxa in Hess samples increased slightly from reference to far-field areas in the Prince George region; numbers of taxa were consistent throughout the Quesnel region. The RCA method resulted in greater numbers of taxa collected at all stations, with highest numbers in Quesnel relative to Prince George. The Hess method collected a higher proportion of Chironomidae per station; the RCA method collected higher proportions of Plecoptera and Oligochaeta.

Statistical Analyses - Hess method:

Near-field and far-field station densities and number of taxa were compared to regional reference area means ± 2 standard deviations (i.e., 95% confidence intervals) (Table 1). No individual station exhibited densities or number of taxa less than reference confidence intervals; several stations exhibited greater densities and/or taxa richness. No significant differences were observed in density or number of taxa among areas using Hess sample data for ANOVA tests (Table 2).

Table 1. Mean station density and total number of taxa (lowest level), relative to reference area mean±2 SD, per region, for Hess samples, upper Fraser River, September 1998.

Prince George	Density	Total Taxa	Quesnel	Density	Total Taxa
Reference Stations			Reference Stations		
FREB1A	1,343	13	FREB5A	517	15
FREB1	1,023	20	FREB5	1,023	18
FREB2	613	14	FREB6	587	16
Ref. mean	993	16	Ref. mean	709	16
Ref. mean±2 SD	261/1,725	8/23	Ref. mean±2 SD	160/1,258	13/19
Near-field Stations			Near-field Stations		
FREB3	1,027	16	FREB7	323	14
FREB3A	1,443	23	FREB7A	777	18
FREB3B	350	15	FREB7B	697	17
FREB4	2,013*	26*	FREB8	713	21*
FREB4A	613	21	FREB8A	1,103	18
FREB4B	363	12	FREB8B	263	14
Far-field Stations			Far-field Stations		
FREB9	713	19	FREB12	987	14
FREB10	1,843*	22	FREB13	577	17
FREB11	953	26*	FREB14	767	14

* Stations exceeding 2 SD of regional reference mean.

Table 2. Results of ANOVA (followed by Tukey's post hoc test) using benthic invertebrate station data to assess pulpmill effluent impacts by reference, near-field and far-field areas, upper Fraser River EEM Cycle 2, September 1998.

Independent Variable	Dependent Variable	F-ratio degrees of freedom	p-Vlaue
All Stations			
Area	Abundance	0.468 (7,16)	0.844
Area	Log_{10} Abundance	0.437 (7,16)	0.865
Area	Number of Taxa	1.178 (7,16)	0.368
Prince George Region			
Area	Abundance	0.076 (3,8)	0.971
Area	Number of Taxa	0.985 (3,8)	0.447
Quesnel Region			
Area	Abundance	0.183 (3,8)	0.905
Area	Number of Taxa	0.646 (3,8)	0.607

Statistical Analyses - RCA:

No significant differences were observed in community structure among baseline reference stations and exposed stations. All stations were clustered with Group 2 reference sites (15 sites in group), indicating no impact (Figs. 1 and 2). No major differences (i.e., no significance levels applied) were observed in taxonomic composition at exposed stations relative to predicted composition from reference stations (Table 3).

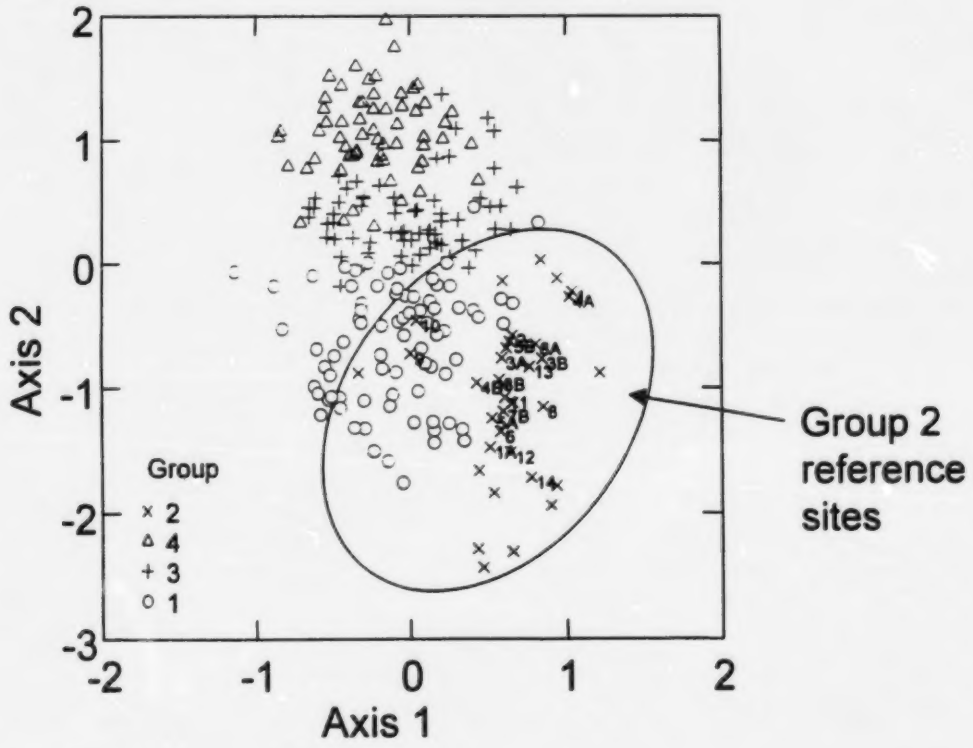

Fig. 1. Fraser River EEM 1998 RCA samples - all sites.

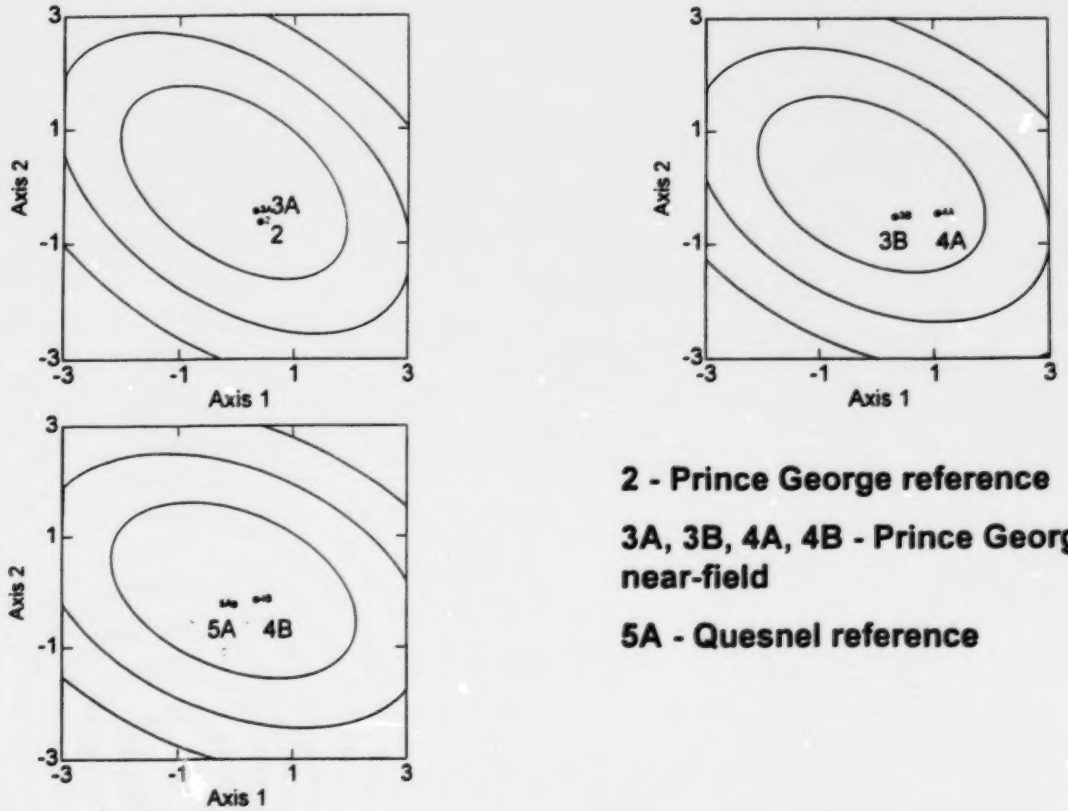

Fig. 2. Fraser River EEM 1998 RCA samples - selected sites relative to Group 2.

Table 3. Assessment of reference, near-field and far-field stations using the RCA relative to reference stations and predicted numbers of families of invertebrates.

Station		Assessment[1]	Families of Predicted	Reference Observed	Invertebrates Missing
Prince George					
Ref	FREB1	1	10	2	Heptageniidae, Baetidae, Ephemerellidae, Nemouridae, Chloroperlidae, Empididae, Perlodidae, Tipulidae
	FREB1A	1	10	6	Baetidae, Nemouridae, Empididae, Tipulidae
	FREB2	1	9	7	Baetidae, Nemouridae
N-F	FREB3A	1	9	6	Nemouridae, Chloroperlidae, Ameletidae
	FREB3B	1	9	6	Baetidae, Nemouridae, Chloroperlidae
	FREB4A	1	10	6	Baetidae, Nemouridae, Chloroperlidae, Tipulidae
	FREB4B	1	10	6	Baetidae, Nemouridae, Chloroperlidae, Tipulidae
F-F	FREB9	1	9	7	Baetidae, Nemouridae
	FREB10	1	9	8	Nemouridae
	FREB11	1	10	8	Baetidae, Nemouridae

Table 3. Continued

Quesnel					
Ref	FREB5A	1	9	7	Nemouridae Chloroperlidae
	FREB5B	1	9	6	Baetidae, Nemouridae, Chloroperlidae
	FREB6	1	11	8	Nemouridae, Chloroperlidae, Tipulidae
N-F	FREB7B	1	10	9	Nemouridae
	FREB8	1	9	6	Nemouridae, Chloroperlidae, Ameletidae
	FREB8A	1	9	7	Nemouridae, Chloroperlidae
	FREB8B	1	9	7	Nemouridae, Chloroperlidae
F-F	FREB12	1	10	6	Baetidae, Nemouridae, Chloroperlidae, Tipulidae
	FREB13	1	9	6	Baetidae, Nemouridae, Chloroperlidae
	FREB14	1	10	8	Nemouridae, Tipulidae

[1]Assessment is based on ordination of sites relative to previously collected reference sites; where, 1 = equivalent to reference; 2 probably different; 3 different; and 4 very different for families indicated as absent based on a >50% probability of occurrence.

CONCLUSIONS

Each method indicated that density/abundance and number of taxa were relatively consistent within each region of the upper Fraser River. The RCA method sampled a larger area of river relative to the Hess sampler, resulting in higher numbers of taxa (genus/ species usually) per station. Statistical analytical results were the same regardless of the method of collection or analysis - no impact of pulpmill effluent was evident downstream of discharges. Either method may be used for future invertebrate surveys of the upper Fraser River. Given no effects were found with either method, it is not possible to assess the effectiveness of methods and analyses in identifying impacts.

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Application of a Community-Based Approach for Monitoring the Health Status of Fish. P.H. Martel, T.G. Kovacs and R.H. Voss. Pulp and Paper Research Institute of Canada, Pointe Claire, QC.

Fish communities of the Saint-François River in Quebec were examined following process changes

and installation of secondary treatment at three pulp and paper mills. The fish assemblages were characterized according to an index of Biotic Integrity (IBI) conducted at 13 locations along a 75 km stretch of the river in 1991 and 1998 using the protocols of the Quebec Ministry of the Environment (QME). Each station was sampled along 500 m of shoreline on both sides of the river by using an electric shocker. The captured fish were identified, measured and weighed. An index of biotic integrity (IBI) was calculated based on metrics such as the number of *Catostomidae* and pollution tolerant species, the percent omnivores, insectivores and carnivores and the proportion of fish with visible anomalies. The seventh metric incorporated abundance and diversity estimates. Based on the total IBI score, fish assemblages were classified from poor to excellent. A total of 5,003 fish were examined representing 28 species, several trophic levels and different degrees of pollution tolerance. In 1991 the QME study showed effluent-related effects downstream from mills not having secondary treatment. In 1998, our follow-up study showed improvements after the installation of secondary treatment at two mills and process modifications/improved effluent treatment at a third mill. The improvements reflected enhancements in effluent quality with respect to BOD, suspended solids and toxicity.

A Collaborative Study Examining Reproductive Function in Wild Fish Downstream of the Fraser Papers Mill in Thurso, Quebec. M.E. McMaster[1], G. Tetreault[1], K.R. Munkittrick[1,2], C. Wood[3] and R. Riffon[4]. [1]Environment Canada, National Water Research Institute, Burlington, ON; [2]Department of Biology, University of New Brunswick, Fredericton, NB; [3]Nexfor, Noranda Technology Centre, Point-Claire, QC; [4]Fraser Papers Inc. Thurso Pulp, Thurso, QC.

Cycle 1 reports for the Fraser Papers Inc. mill at Thurso (hardwood, bleached kraft), Quebec, suggested some reproductive responses in feral fish were evident downstream of the discharge. Fish sampled from the mill plume at Thurso indicated a reduction in ovarian weights in brown bullhead and an increase in liver weights in male yellow perch. At this time, Fraser Papers and Environment Canada signed a memorandum of agreement to undertake the following research study as an alternative to the fish survey of the EEM program for Cycle 2. The objectives of this research study were to: [1] further clarify and validate the findings of Cycle 1; [2] analyze reproductive health of fish populations; and [3] evaluate the installation of secondary treatment system; and [4] assess the potential for the use of forage fish in the EEM program. Overall, results from the two years of study at the Thurso location answered a number of relative EEM questions. The reproductive alterations found during Cycle 1 were not evident during either year of the research program. Other alterations found during 1997 were not present in 1998 following a number of process changes at the mill. Our studies also indicate that determining the magnitude and the extent of the alterations in fish populations to mill effluent is very critical in the EEM program as is the selection of reference sites. Our in depth studies with forage fish demonstrated that fish less than 1 g can be utilized for EEM studies as they may be less mobile than some of the larger species used during the first cycle of the EEM program.

Does Exposure to Retene Cause Oxidative Stress in Larval Trout? M.B. Bauder, C. Picco, S.M. Billiard, J. Waller and P.V. Hodson. Queen's University, Kingston, ON.

Blue sac disease (BSD) of larval rainbow trout follows chronic exposure to chlorinated dioxins and furans, PCBs, and retene, an alkyl-substituted phenanthrene. The symptoms of BSD include yolk sac and pericardial edemas, haemorrhaging, craniofacial deformities, induction of mixed function oxygenase (MFO) enzymes, and mortality before swim-up. A proposed mechanism of toxicity is the generation of oxyradicals and/or reactive metabolites as a result of increased MFO activity and transformation of aromatic substrates. Symptoms of edema and haemorrhaging are consistent with leakiness of endothelial membranes due to lipid peroxidation, and the edematous fluid in the yolk sac

appears to be an ultra-filtrate of blood. To test this hypothesis, we measured biochemical indices of oxidative stress in juvenile trout exposed to paraquat, a pro-oxidant (positive control), and in larval trout exposed to concentrations of retene that were sufficient to cause symptoms of BSD. Our preliminary tests with measures of thiobarbituric reactive substances (TBARS) and of lipid peroxides demonstrated clear effects of paraquat exposure, but no effects of retene exposure. These results suggest that either oxidative stress is not the mechanism of toxicity of retene or BSD in general, or that the indices used were not adequate. Our current work includes measurement of the antioxidant glutathione.

Species Variation in Toxicity of Retene to Embryos and Larvae of Teleosts. S.M. Billiard[1], Y. Kiparissis[1], J. Reynolds[1], K. Querbach[2], A. Winchester[1], N. Fragoso[1], A. Oikari[3] and P.V. Hodson[1]. [1]School of Environmental Studies, Queen's University, Kingston, ON; [2]Dalhousie University, Halifax, NS; [3]University of Jyväskylä, Jyväskylä, Finland.

Retene, an alkyl substituted phenanthrene derived from the bacterial aromatization of abietic acid, is enriched up to 20,000-fold in sediments downstream of effluent discharges from pulp mills using softwood furnish. We have used embryo-larval bioassays to assess the toxicity of retene to early life stages of fish. Rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Danio rerio*), Japanese medaka (*Oryzias latipes*), blue gill (*Lepomis macrochirus*), walleye (*Stizostedion vitreum*), and herring (*Clupea pallasii*) embryos were exposed in semi-static renewal bioassays to waterborne retene (10, 32, 100, 320 and 1000 µg/L) from fertilization to "swim-up." Exposure to retene caused blue sac disease (BSD) in all test species, characterized by circulatory abnormalities (haemorrhage, blood stasis), pericardial and yolk sac edemas, craniofacial and skeletal deformities, unsuccessful swim bladder inflation, and mortality before feeding begins. The threshold concentrations of waterborne retene for BSD were species-dependent. Based on the nominal threshold concentration of retene causing an increased prevalence of BSD, the sensitivity of fish species to retene exposure could be ranked as: walleye = herring (10 µg/L) > blue gill = rainbow trout (32 µg/L) > medaka (100 µg/L) > zebrafish (320 µg/L). The sensitivity of a wide range of species to retene exposure may help to explain observations of recruitment failure of fish species near European pulp mills.

The Effect of Polycyclic Aromatic Hydrocarbon Biotransformation on Early Life Stage Toxicity in Rainbow Trout (*Oncorhynchus mykiss*). S.A. Hawkins, S.M. Billiard, S.P. Tabash, D.C. Depew, R.S. Brown and P.V. Hodson. School of Environmental Studies, Queen's University, Kingston, ON.

The polycyclic aromatic hydrocarbons (PAHs) retene (1-methyl-7-isopropyl phenanthrene) and phenanthrene are lethal to rainbow trout (*Oncorhynchus mykiss*) larvae during chronic exposures. Phenanthrene is a low toxicity, non-mixed function oxygenase (MFO)-inducing compound that accumulates in fish tissues during lethal exposures. Retene is a higher toxicity MFO-inducing compound that is not detectable in tissue at lethal exposure concentrations. Therefore, the role of MFO enzyme activity in the metabolism, excretion, and toxicity of retene and phenanthrene in juvenile trout was examined. Larval and juvenile trout were exposed to the MFO inducer - naphthoflavone (NF) or the inducer-inhibitor piperonyl butoxide (PBO), plus retene or phenanthrene. Changes in metabolism and excretion of PAHs were determined by analyses of parent compound and metabolites in tissues and bile using MFO assays, HPLC, and fluorescence. Co-exposures of juvenile trout to NF increased phenanthrene, but not retene, metabolism and excretion, whereas PBO co-exposure inhibited MFO-mediated metabolism and excretion of both retene and phenanthrene. These results support the role of MFO activity in PAH metabolism and excretion, although certain metabolites were produced independently of induced MFO activity. Changes in early life stage toxicity in larval trout are currently being determined; the results of this testing, and its implications for the mode of PAH toxicity, will be discussed.

Potential of the Flavonoid, Genistein to Alter Gonadal Development in Fish. Y. Kiparissis[1], R. Hughes[1], C.D. Metcalfe[1], A.J. Niimi[2] and T. Ternes[3]. [1]Trent University, Peterborough, ON; [2]Department of Fisheries and Oceans, Burlington, ON; [3]ESWE Institute for Water Research and Water Technology, Wiesbaden, Germany.

Fish exposed to pulp and paper mill effluents experience a multitude of adverse reproductive effects. The active compounds in these effluents have not been positively identified. Plants synthesize many bioactive secondary metabolites, such as flavonoids, with endocrine-modulating potential. Bleached kraft mill effluent (BKME) samples collected from a pulp mill in Espanola, Ontario were analyzed by LC-ESI-MS and LC-ESI-MS-MS. The analytical data indicated the presence of genistein, an isoflavonoid, and a monohydroxyflavone in µg/L concentrations in the final effluent. Exposure of Japanese medaka (*Oryzias latipes*) to genistein at concentrations of 1,10, 100 and 1000 µg/L for 3 months altered gonadal development. Specifically, histological evaluation revealed inhibition of spermatogenesis in male medaka and the development testis-ova (intersex) at the highest concentration. In addition, the expression of secondary sex characteristics was altered or reduced in a concentration dependent manner. It is possible, that these reproductive effects are mediated through the binding of genistein to the estrogen receptor, since genistein gave a positive response in the yeast estrogenicity screening (YES) assay. However, it is also possible that genistein affects development of fish through other mechanisms besides estrogenicity. These results indicate that flavonoid compounds have the potential to contribute to the reproductive and developmental effects observed in feral fish exposed to pulp mill effluents.

Bioremediation

Evaluation of a Method for *In Situ* Exposures with Fish Eggs in the Intertidal Zone. A. Gagné, P. Robichaud, J. Gauthier, N. Desrosiers and R.L. Roy. Pêches et Océans Canada, Institut Maurice-Lamontage (IML), Mont-Joli, QC.

We developed an apparatus for exposing eggs of estuarine fish to contaminated sediments. Fertilized eggs are exposed in a system consisting of High-Density Polyethylene panels with incubation chambers for the eggs. The panels are protected with screens of 350-µm Nitex mesh. The system was tested in the laboratory and the field with eggs of the mummichog (*Fundulus heteroclitis*) and the three-spined stickleback (*Gasterosteus aculeatus*). One field trial included exposures to oiled sediments. Egg survival and hatching success was acceptable following laboratory trials with mummichogs and field trials with sticklebacks. Egg survival in field studies depended on the source of eggs (captured vs laboratory animals) and on the method used to obtain fertilized eggs (laboratory cultures vs manual stripping). Exposure to oiled sediments resulted in complete mortality of mummichog eggs in a relatively short period of time. The results of exposures at control sites indicate that sediment grain size and the presence of parasites adversely affect egg development and survival.

Polycyclic Aromatic Hydrocarbons in Invertebrates of Black Duck Cove, Nova Scotia: 30 Years After a Bunker C Fuel Oil Spill. J. Hellou[1], C. Anstey[1], T. King[1], J. Leonard[1], S. Steller[1], S. Cobalni[1] and K. Lee[2]. [1]Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS; [2]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC.

Black Duck Cove, Nova Scotia was heavily impacted by a spill of Bunker C fuel oil in 1970 following the grounding of the tanker *Arrow*. Habitat recovery has been observed at this site and the results of recent sediment and interstitial water biotests has confirmed that the toxicity of residual oil in the sediments is low. To provide further evidence on the extent of site recovery, representative samples of the common periwinkle, *Littorina littorea*, the amphipod, *Gammerus oceanicus*, and the soft-shelled

clam, *Mya areneria*, were recovered from three distinct eco-habitats found within the cove. At each of the sites, periwinkles displayed the highest PAH concentrations (wet weight), while concentrations in amphipods were relatively similar to clams. However, periwinkles also displayed five-times lower moisture content than the other two species, explaining some of the observed differences. The bioavailability of PAHs differed with species due to their preferred habitat and manner of feeding. In general, for the invertebrate samples collected, background levels of combustion PAHs were observed with a possibility of traces of Bunker C fuel oil observed in clams.

The Response of *Scirpus pungens* to Crude Oil Contaminated Sediments. D. Longpré[1], K. Lee[2], G.H. Tremblay[2] and V. Jarry[3]. [1]University of Quebec at Montreal, Montreal, QC; [2]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC; [3]Environment Canada, Montreal, QC.

The wetland plant *Scirpus pungens* is commonly found along the shores of the St. Lawrence River. To fully assess the impact of an accidental oil spill in wetlands dominated by this ecologically-important species, both plants and sediments were recovered from a site near Ste. Croix, Quebec for controlled contaminant exposure studies with a weathered medium-light crude oil. Transplants in oiled (range: 1.14–72.9 g/kg) and unoiled (control) sediments under simulated wetland conditions were monitored over a 63 d period time to quantify changes in plant height, new growth, and mortality. Plants exposed to high concentrations of oiled sediment (36.5–72.9 g/kg) were significantly smaller, grew more sparsely and had a higher mortality rate than those exposed to control and/or lightly-contaminated sediments (<4.56 g/kg). Sediment samples representing each experimental treatment were analysed by gas chromatography/mass spectroscopy. Under the current test conditions, in which nutrients were not limited, significant enhancement of hydrocarbon degradation by the growth of the plants (phytoremediation activity) was not observed. Study results indicate that transplants of *Scirpus pungens* were able to survive, grow, and produce new shoots in sediments contaminated with crude oil in a range of concentrations comparable to those associated with oil spill incidents.

A Microscale Test to Measure Petroleum Oil Toxicity to Marine Fish Embryos. C.M. Couillard[1], A. Weise[1], H. Dionne[1] and S. Briatte[1]. [1]Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli, QC.

A new assay was developed to compare the toxicity of different petroleum oils to mummichog (*Fundulus heteroclitus*) embryos. Fertilized eggs were incubated during 11 d at 22°C directly on the surface of oil-contaminated sand in 47 mm glass petri dishes without a superficial water layer. Six serial dilutions ranging from 100% to 2% v/v petroleum oil in mineral oil were prepared. In each petri dish, 0.5 ml of each dilution was mixed with 12 g of sand and 3 ml of filtered seawater. At the end of the incubation, the mortality rates, the hatching rates, the body length and the prevalence of malformations were determined. The toxicity of two weathered petroleum oils, an Alaska North Slope crude oil (ANCO) and a Mesa light crude oil (MLCO), were compared. Both oils produced similar symptoms of toxicity including retarded growth and development, reduced hatching rates, pericardial edema, cardiac malformations, hemorrhages and spinal deformities. However, the MLCO was consistently more embryotoxic than the ANCO. Body length was inversely correlated to oil concentration in embryos exposed to both oils but at the highest concentration tested, body length was 2 times lower in embryos exposed to MLCO compared to those exposed to ANCO. This assay will be used to evaluate the effects of weatherisation or of bioremediation on petroleum oil toxicity.

Emerging Issues and Application of New Technologies

Utilization of a Human Liver Cellular Bioassay to Assess Microcyctin-LR Toxicity. A.M. Carr and P.F. Dehn. Department of Biology, Canisius College, Buffalo, NY.

Cyanobacterial contamination of water supplies causes serious public health problems world-wide. HepG2 cells were exposed to 0.05–50 μM of purified Microcystin-LR (MYST) for 24 or 72 h, after which cell viability, membrane damage, phase II detoxification capabilities (glutathione conjugation), oxidative metabolism, and apoptosis were examined. At least 3 replicate experiments with a minimum of 6 replicates per assay were run. Mann-Whitney Rank Sum tests (α 0.05) compared solvent control (SC) and MYST exposed groups. No toxic responses were observed at concentrations lower than 50 μM or before 72 h of exposure. MYST significantly: [1] reduced viability (39.5% for neutral red, 30.5% for MTT); [2] increased lactate dehydrogenase (LDH) leakage (277%); [3] depleted glutathione (GSH) (56.3%); [4] increased ATP levels (408%); and [5] increased apoptotic activity (251%) when compared to the SC group. These data suggest that: [1] GSH offers protection against MYST, as no GSH depletion occurred below 50 μM exposure levels and no cytotoxic effects were observed until GSH levels declined; [2] MYST does have an impact on the cell membrane, as LDH leakage did increase; [3] the toxin does affect energy metabolism as ATP levels increase; and [4] MYST increases apoptotic activity in this cell line. Of note however, is MYST's delay in impact on this cell line, as no effects are seen until 72 h after initial exposure. It appears that the HepG2 cell line may be a suitable *in vitro* human model for assessing Microcystin-LR toxicity. (AMC supported by Merck/AAAS USRP award).

An Investigation of the Degradative Abilities of Selected Psychrotrophic Bacteria Isolated from a PCB Contaminated Site in Argentia, Newfoundland. D. Squires-Parsons and T.R. Patel. Department of Biology, Memorial University of Newfoundland, St. John's, NF.

Sediments from Shag Pond, Argentia, Newfoundland, were used for the isolation and characterization of several strains of aerobic bacteria capable of biodegrading numerous aromatic and polyaromatic hydrocarbons, such as naphthalene, phenanthrene, phloroglucinol and toluene. The isolates included aerobic and facultatively anaerobic strains, mostly from the Gram negative group. Only one Gram positive strain was isolated. Work is in progress to establish their ability to attack PCB congeners.

Chlorobiphenyls From a Non-Aroclor Source. Where do They Come From? T. King, P. Yeats, J. Hellou and S. Niven. Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS.

Chlorobiphenyls (CBs), which appear to have a non-Aroclor origin, have been found in mussels collected in Halifax Harbour, Nova Scotia. Analysis of samples for 159 CBs (resolved and co-eluting pairs) revealed the presence of 3,3'-dichlorobiphenyl (CB 11) found in water, suspended particulate matter (SPM), and mussels in two recent studies of Halifax Harbour. In the first of these, water and SPM samples were collected at a single site in Bedford Basin weekly over the duration of the spring phytoplankton bloom in 1996 and 1997. In the second, mussels were collected at 17 sites around the shoreline of the harbour on three occasions (Spring, Summer, and Fall) between July 1997 and April 1999. Our observations indicate that CB 11 is a dominant peak in the CB spectrum of water (4-14% of total CB concentrations), SPM (0.6-3.1% of total CB concentrations) and mussels (in most cases <20% of the summed CB concentrations). CB 11 has also been found in lobsters (earlier study on the harbour) and sediment (in some cases 10% of summed CB concentrations in sediment) collected during the bloom study. The focus of this presentation will be on using results from the two studies and some additional measurements of lobsters and sediments to compare the non-Aroclor CB profiles and investigate the geographical origin and source of these CBs.

Identification of Changes in Gene Expression in Copper-Exposed *Daphnia magna* Using Differential Display Polymerase Chain Reaction. L.C. Diener, D.G. Dixon, B.M. Greenberg and P.M. Schulte. Department of Biology, University of Waterloo, Waterloo, ON.

Contamination of aquatic systems by metals and polycyclic aromatic hydrocarbons (PAHs) is a prevalent environmental problem. These contaminants are known to negatively impact populations, organism health, and survival. Most of the organism and ecosystem level changes are a consequence of underlying molecular and subcellular damage. Therefore, molecular bioindicators are likely to be a sensitive tool for environmental assessment. We have demonstrated that both Cu and phenanthrenequinone (PHEQ) alter protein expression in *Daphnia magna*. To investigate altered gene expression in *D. magna* exposed to Cu, PHEQ, and other contaminants, a technique based on the differential display polymerase chain reaction (ddPCR) is being developed for *D. magna*. This technique promises numerous applications as it permits a survey of the genes being expressed in any given organism. Furthermore, ddPCR allows one to monitor the changes in gene expression that result from any toxicant exposure. *D. magna* were exposed to increasing doses of copper sulfate for 48 h. The copper exposure resulted in changes in the expression of a variety of different genes. These results demonstrate that ddPCR can be used to identify numerous genes that are simultaneously affected by exposure to a toxicant.

Use of Effects of Polycyclic Aromatic Hydrocarbons on the Synaptosomal Membrane Permeability as a Biomarker. P. Eghtesadi-Araghi[1,2], G. Riazi,[1], A. Mohebbi[1], O. Ranaiee-Saidat[1], H. Zomorrodian[2] and M.R. Sheikoleslami[2]. [1]Institute of Biochemistry and Biophysics(IBB), University of Tehran, Tehran, Iran; [2]Iranian National Center for Oceanography (INCO), Tehran, Iran.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous petroleum-derived marine pollutants composed by fusion of several rings of benzene rings; they are lipophilic and toxic to biological organisms. The primary site of the toxic action is probably the membrane and PAHs in this manner affect the structure and function of membranes. The lipophilicity of PAHs make them prone to accumulate in the tissues of higher fat content e.g., the nervous system; hence synaptosomes (pinched-off nerve endings) has been used to determine these effects on biological membranes. In this study we sampled the sediments of the Persian Gulf along the northern coast from east to the west in order to obtain an estimation of the pollution in this area and also the concentration of PAHs for *in-vitro* studies by GC/FID. The accumulation of three selected PAHs (anthracene, phenanthrene and pyrene) in synaptosomal membranes resulted in an increase in membrane permeability (increased passive flux of water) which was assessed by light scattering. These results suggest a possible mechanism of toxic action of PAHs on synaptosomal membranes through accumulation in the membrane and the potential use of this parameter as a biomarker for PAHs.

Selection and Optimisation of New Immune Function Assays for the Tiger Salamander (*Ambystoma tigrinum*). J.M. Willson[1], J.E.G. Smits[1], D.J. Forsyth[2] and M.L. Wickstrom[1]. [1]Toxicology Centre, University of Saskatchewan, Saskatoon, SK; [2]Canadian Wildlife Service, Saskatoon, SK.

North American amphibian populations have been declining for several decades. The cause of this decline is unknown but is likely a combination of various factors. Proposed explanations include local climate change, acid precipitation, increased ultraviolet irradiation, increased incidence of infectious disease and environmental pesticide contamination. The current study combines two of these factors in an evaluation of how the immune system of tiger salamanders *(Ambystoma tigrinum)* is affected by exposure to commonly used pesticides. As a group, urodele amphibians have a unique immune system, such that the standard tests used to assess immune function have proven inadequate. Therefore, our first research objective was to adapt existing immune assays for use with this species.

Technique optimisation is currently underway for a lymphocyte blastogenesis assay, a plaque assay, a peritoneal macrophage phagocytosis assay, a delayed-type hypersensitivity assay and differential white blood cell counts. When complete, these assays will provide us with the tools to assess the immunotoxicity of pesticides and increase our understanding of the interaction between pesticide use and incidence of infectious disease in amphibian populations.

Fate and Behavior of Metals Released from Urban Wastewater Effluents. C. Gagnon and I. Saulnier. Environment Canada, Centre Saint-Laurent, Montreal, QC.

Municipal wastewater effluents are major threat to the aquatic environment. The impacts of urban wastewater discharge on receiving waters are numerous and inputs of contaminants such as metals can cause toxicity of organisms in receiving waters, as they may be incorporated in the biota. The biological availability of metals is determined by their speciation in both the dissolved and particulate phases, which in turn is strongly influenced by the physico-chemical conditions of the environment. The objective of this study was to determine the chemical forms of metals such as Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Si, Zn in the receiving waters of a major urban effluent discharging to the St. Lawrence River. Surface water, suspended matter and sediments were sampled at several sites, 0.5-10 km downstream of a municipal outfall plume in the river. Total and extractable metal concentrations were determined by ICP-ES/MS and AAS. Physico-chemical parameters such as pH, Eh, turbidity, SPM, and dissolved and particulate organic carbon were also measured to characterize the receiving waters and evaluate their influence on the fate and behavior of metals released from the urban effluent. This study on metal speciation provides key information for the determination of bioavailability of metals to aquatic organisms and the assessment of toxic potential in the receiving waters.

Selection Criteria for Chemicals Considered as a Biocide to Treat Ballast Water for Exotic Aquatic Organisms. A.J. Niimi. Department of Fisheries and Oceans, Burlington, ON.

Accidental introductions of exotic organisms through the discharge of ballast water by overseas vessels is a continuing global problem because of their negative economic and environmental impacts in many major aquatic ecosystems. Various treatment options are currently under consideration that could have a chemical, mechanical and physical basis. Use of a chemical biocide appears to the most promising to meet the urgent need for an interim treatment because no proactive methods are currently available. A large information base is available on the toxicity of many chemicals to organisms that range from microbes to fish. Volumes of ballast water treated can range from tens to several thousand cubic meters. Selection of chemicals would be guided by several underlying concerns. Safety to vessel crew and treatment applicators, and adverse effects on the structural, mechanical and operational integrity of the vessel, are primary limiting factors in chemical selection. Desirable properties of a chemical would include information on its environmental fate; readily soluble in water; short chemical persistence; low surface adsorption; and minimal effect of environmental conditions on toxicity. The large taxonomic range of organisms encountered would likely require the use of several chemicals with different modes-of-actions to be applied as a mixture, therefore low interaction among chemicals would be a desirable property. Regulatory approval may be required to allow some flexibility in use of chemicals in this specialization application whose goal is to prevent exotic introductions. The probability for eradicating an exotic organism is virtually nil once it has become established in a large aquatic ecosytem.

Mixed Function Oxygenase Activity of Rainbow Trout (*Oncorhynchus mykiss*) in Relation to Specific Developmental Stages. L.C. Brinkworth, S.M. Billiard, Y. Kiparissis and P.V. Hodson.

Department of Biology and School of Environmental Studies, Queen's University, Kingston, ON.

Previous studies indicate that rainbow trout embryos (*Oncorhynchus mykiss*) exposed to retene (1-methyl-7-isopropyl phenanthrene) or phenanthrene from 21-42 d post fertilization display a variety of defects prior to swim up. These include developmental delays, pericardial edema, circulatory anomalies, and craniofacial malformations, all of which are symptoms of bluesac disease. The purpose of this study was to evaluate which stages of embryonic development are sensitive to the toxic effects of polycyclic aromatic hydrocarbons by analyzing mixed function oxygenase induction. If induction is part of the mechanism we expect that toxicity will occur when Cyp 1A1 first becomes activated. We are testing this hypothesis by exposing trout from fertilization to swim-up and comparing prevalence of symptoms of toxicity (bluesac disease) to the activity of Cyp 1A1 as indicated by immunohistochemistry using an antibody to the Cyp 1A1 protein.

Controlled Release of Polycyclic Aromatic Hydrocarbons from Polydimethylsiloxane Films to Solutions in Aqueous Toxicity Tests. P. Akhtar[1], R.S. Brown[1], J.C. Klamer[2] and F. Smedes.[2] [1]Department of Chemistry and School of Environmental Studies, Queen's University, Kingston, ON; [2]RIKZ (Institute of Coastal and Marine Management), Haren, The Netherlands.

In aqueous toxicity tests involving insoluble and very nonpolar compounds, the actual test concentration could be different from the "nominal" concentration because adsorption, volatilization, precipitation, or other processes cause depletion. Therefore, a reliable method is required to deliver and maintain a constant exposure level of toxicant in the test solution. We have developed toxicant-loaded polydimethylsiloxane (PDMS) films to produce controlled aqueous concentrations by partition-controlled delivery. Films containing various levels of several polycyclic aromatic hydrocarbons (PAHs) were deposited in 96-well microtitreplates. Equilibrium with a solution in the microwell required 30 min under constant shaking producing a solution concentration which was proportional to the film concentration. The film-solution partition constant (K_{fs}) was determined, and films were stable over a period of two weeks. Tests with anthracene provided solutions with 6.3×10^{-9}M - 1.6×10^{-7}M. In a mixture with excess naphthalene, fluorene, phenanthrene, fluoranthene and pyrene, the anthracene K_{fs} value of 28,000 was not affected. The films were used to deliver PAHs to Microtox® and Mutatox® tests, and EC_{50} values were determined for comparison with literature values.

Are Road Salts Toxic? Priority Substances List Assessment and Follow-Up Actions. R. Chénier. Environment Canada, Commercial Chemicals Evaluation Branch, Hull, QC.

The environmental impacts of road salts were assessed under the *Canadian Environmental Protection Act* Priority Substances List (CEPA PSL) program. Road salts include inorganic chloride salts (NaCl, CaCl, KCl, MgCl) and ferrocyanides. Because of the environmental risks related to the very large amounts released (about 5 million tonnes per year), it is proposed that road salts be added to the CEPA List of Toxic Substances and that control instruments be developed. Key environmental concerns relate to impacts on freshwater ecosystems, resulting both from direct contamination of surface water and from movement of chloride ions through soil and groundwater. Impacts are also associated with terrestrial vegetation, soils, and wildlife. Transformation of ferrocyanide to release cyanide can pose a risk to aquatic vertebrates. Key assessment findings will be summarized and the research and management implications discussed.

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BEST STUDENT PAPER AWARDS/
PRIX POUR LES MEILLEURS EXPOSÉS PAR DES ÉTUDIANTS

PETRO-CANADA BEST PLATFORM PAPER AWARD

Graham Sherwood
Department of Biology, McGill University, Montreal, QC

Simplified Prey Communities Lead to Energetic Bottlenecks for Yellow Perch Inhabiting Metal-Contaminated Lakes.

PETRO-CANADA BEST POSTER PAPER AWARD

Deborah Squires-Parsons
Department of Biology, Memorial University of Newfoundland, St. John's, NF

Biodegradation of PCBs from Contaminated Sites by Selected Psychrotrophic Organisms Isolated Locally.

2nd Place Platform Presentation:
Ken Oakes, Department of Zoology, University of Guelph, Guelph, ON

3rd Place Platform Presentation:
Melissa Schwartz, Department of Biology, Wilfrid Laurier University, Waterloo, ON

2nd Place Poster Presentation:
Lara Diener, Department of Biology, University of Waterloo, Waterloo, ON

3rd Place Poster Presentation:
Lyndon Brinkworth, Department of Biology, Queen's University, Kingston, ON

WORKSHOP PROCEEDINGS/COMPTE RENDUS D'ATELIER

The Proceedings of each Annual Aquatic Toxicity Workshop have been published in a series of Technical Reports listed below. These Proceedings are generally provided to each Workshop participant, and are also sent to selected libraries, government departments and other agencies. Copies of 4th and subsequent Proceedings may be available for a charge, as photocopies or fiche, from Micromedia Limited, 240 Catherine Street, Suite 305, Ottawa, ON, K2P 2G8 (613-237-4250).

Proceedings of the 26th Annual Aquatic Toxicity Workshop: October 4-6, 1999, Edmonton, Alberta. Edited by E.G. Baddaloo, M.H. Mah-Paulson, A.G. Verbeek and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2293: 155 p.

Comptes rendus du 25e colloque annuel de toxicologie aquatique: 18-21 octobre 1998, Québec, Québec. Éditeurs: R. Van Coillie, R. Chassé, C. Julien, L. Martel, C. Thellen et A.J. Niimi, M.D. Treissman and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2260: 134 p.

Proceedings of the 24th Annual Aquatic Toxicity Workshop: October 19-22, 1997, Niagara Falls, Ontario. Edited by A.J. Niimi, G.L. Parrott and D.G. Spry. Can. Tech. Rep. Fish. Aquat. Sci. 2192: 135 p.

Proceedings of the 23rd Annual Aquatic Toxicity Workshop: October 7-9, 1996, Calgary, Alberta. Edited by J.S. Goudey, S.M. Swanson, M.D. Treissman and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2144: 196 p.

Proceedings of the 22nd Annual Aquatic Toxicity Workshop: October 2-4, 1995, St. Andrews, New Brunswick. Edited by K. Haya and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2093: 159 p.

Proceedings of the 21st Annual Aquatic Toxicity Workshop: October 3-5, 1994, Sarnia, Ontario. Edited by G.F. Westlake, J.L. Parrott and A.J. Niimi. Can. Tech. Rep. Fish. Aquat. Sci. 2050: 179 p.

Proceedings of the 20th Annual Toxicity Aquatic Workshop: October 17-21, 1993, Quebec City, Quebec. Edited by R. Van Coillie, Y. Roy, Y. Bois, P.G.C. Campbell, P. Lundahl, L. Martel, M. Michaud, P. Riebel and C. Thellen. Can. Tech. Rep. Fish. Aquat. Sci. 1989: 331 p.

Proceedings of the 19th Annual Aquatic Toxicity Aquatic Workshop: October 4-7, 1992, Edmonton, Alberta. Edited by E.G. Baddaloo, S. Ramamoorthy and J.W. Moore. Can. Tech. Rep. Fish. Aquat. Sci. 1942: 489 p.

Proceedings of the 18th Annual Aquatic Toxicity Workshop: September 30-October 3, 1991, Ottawa. Ontario. Edited by A.J. Niimi and M.C. Taylor. Can. Tech. Rep. Fish. Aquat. Sci. 1863: 381 p.

Proceedings of the 17th Annual Aquatic Toxicity Workshop: November 5-7, 1990, Vancouver, British Columbia. Edited by P. Chapman, F. Bishay, E. Power, K. Hall, L. Harding, D. McLeay, M. Nassichuck and W. Knapp. Can. Tech. Rep. Fish. Aquat. Sci. 1774: 1213 p.

Proceedings of the 15th Annual Aquatic Toxicity Workshop: November 28-30, 1988, Montreal, Quebec. Edited by R. Van Coillie, A.J. Niimi, A. Champoux and G. Joubert. Can. Tech. Rep. Fish. Aquat. Sci. 1714: 244 p.

Proceedings of the 14th Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Edited by A.J. Niimi and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1607: 201 p.

Proceedings of the 13th Annual Aquatic Toxicity Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p.

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Proceedings of the 9th Annual Aquatic Toxicity Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mckay. Can. Tech. Rep. Fish. Aquat. Sci. 1163: 243 p.

Proceedings of the 8th Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 255 p.

Proceedings of the 7th Annual Aquatic Toxicity Workshop: November 5-7, 1980, Montreal, Quebec. Edited by N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert and M. Speyer. Can. Tech. Rep. Fish. Aquat. Sci. 990: 519 p.

Proceedings of the 6th Annual Aquatic Toxicity Workshop: November 6-7, 1979, Winnipeg, Manitoba. Edited by J.F. Klaverkamp, S.L. Leonhard and K.E. Marshall. Can. Tech. Rep. Fish. Aquat. Sci. 975: 291 p.

Proceedings of the 5th Annual Aquatic Toxicity Workshop: November 7-9, 1978, Hamilton, Ontario. Edited by P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns and U. Borgmann. Fish. Mar. Ser. Tech. Rep. 862: 342 p.

Proceedings of the 4th Annual Aquatic Toxicity Workshop: November 8-10, 1977, Vancouver, British Columbia. Edited by J.C. Davis, G.L Greer and I.K. Burtwell. Fish. Mar. Ser. Tech. Rep. 818: 211 p.

Proceedings of the 3rd Annual Aquatic Toxicity Workshop Held in Halifax, Nova Scotia, November 2-3, 1976. Edited by W.R. Parker, E. Pessah, P.G. Wells and G.F. Westlake. Environment Canada, Surveillance Rep. EPS-5-AR-77-1.

Proceedings of the 2nd Annual Aquatic Toxicity Workshop, November 4-5, 1975, Rexdale, Ontario. Edited by G.R. Craig. Ontario Ministry of the Environment.

Compendium of Aquatic Toxicity Studies in Canada. 1974. Unpublished Report, Freshwater Institute, Winnipeg, Manitoba. 39 p. + appendices.